**Universidade do Minho**
Escola de Engenharia

Margarida Barbosa Pereira de Lemos

# Ageing profiling of commercial and craft beers: a sensorial and chemical overview

outubro de 2014

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# Ageing profiling of commercial and craft beers: a sensorial and chemical overview

Dissertação de Mestrado
Mestrado Integrado em Engenharia Biológica
Ramo Tecnologia Química e Alimentar

Trabalho efetuado sob a orientação da
**Professora Doutora Lucília Domingues**
e coorientação do
**Engenheiro Filipe Macieira**

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## RESUMO

A cerveja é uma bebida obtida por fermentação alcoólica que contem álcool, extrato e dióxido de carbono. É preparada a partir de malte de cereais, lúpulo, água e leveduras (a partir dos quais derivam as influências predominantes dos diferentes tipos de cerveja).

Uma vez que os diferentes tipos de cervejas resultam da combinação e relação entre vários fatores (ingredientes, processamento, embalagem, marketing, cultura) o aroma final será diferente para cada uma delas. No entanto, o aroma não é estático mas em estado constante de mudança. O ponto em que a maturação acaba e a deterioração começa é sem dúvida diferente para diferentes cervejas e provavelmente diferente para cada consumidor.

O objetivo deste trabalho foi estudar as alterações que ocorrem durante o armazenamento/envelhecimento de seis cervejas diferentes: quatro artesanais (Weiss, Pilsner, Stout, Red Ale) e duas comerciais (Weiss e Pilsner). As principais diferenças entre estas cervejas é o facto de as artesanais serem feitas exclusivamente a partir de matéria-prima natural, não serem adicionados aditivos nem conservantes e não ser pasteurizada nem filtrada (contendo a levedura na garrafa).

As cervejas foram analisadas sensorial e quimicamente (compostos maioritários e minoritários) uma vez por mês ao longo de seis meses. Os compostos minoritários foram analisados no primeiro e no último mês.

As cervejas artesanais mostraram um perfil aromático muito mais intenso do que as comerciais e mantiveram o perfil constante ao longo dos seis meses de armazenamento (assim como as comerciais). Os resultados permitiram concluir que as cervejas artesanais mantêm a qualidade de uma cerveja comercial, ao longo de seis meses, com a vantagem de terem os sabores e aromas mais intensos.

A análise dos compostos maioritários não permitiram determinar tendências claras acerca da concentração de etanol e de açúcares. As concentrações de ácidos orgânicos mostraram ser mais elevadas do que as concentrações tipicamente encontradas nas cervejas comerciais.

Os resultados da análise dos compostos minoritários vão de encontro aos perfis aromáticos obtidos pela análise sensorial assim como os retratados na literatura. Estes resultados mostraram que a maioria dos principais marcadores de maturação reportados não foram encontrados nas cervejas, no entanto outros compostos foram encontrados tas como álcoois superiores, cetonas e ácidos.

Foi realizada a validação do método de extração de compostos minoritários, utilizado para a análise por Cromatografia Gasosa- Espectrometria de Massa. De forma a validar o método foram estudados vários parâmetros: linearidade, sensibilidade, limites de deteção e quantificação, precisão (precisão intermédia e repetibilidade), efeito matriz e efeito do tempo e exatidão (teste de recuperação). Os resultados mostraram que o método satisfaz as especificações determinadas para cada parâmetro de validação, o que significa que a validação do método de extração foi um sucesso.

# ABSTRACT

Beer is a beverage obtained by alcoholic fermentation, containing alcohol, extract and carbon dioxide. It is prepared from barley malt, hops, brewing water, and yeasts (from which derive the predominant influences on overall beer types).

Since different beer styles result from the combination and relationships between several factors (ingredients, processing, packaging, marketing and culture), the final flavor will be different. However, the beer flavor is not static but in a continuous changing state. The point where maturation ends and deterioration begins is undoubtedly different for different beers and probably different for each consumer.

The aim of this study was to investigate the changes that occur during the storage/ageing of six different of beers: four craft (Weiss, Pilsner, Stout, Red Ale) and two commercial beers (Weiss and Pilsner). The main differences between these beers are the fact that craft beers are made exclusively of natural raw material, no preservatives or additives are added and are they are not pasteurized or filtered (containing the yeast in the bottle).

The beers were analyzed sensory and chemically (major and minor compounds) once a month over six months. Minor compounds were analyzed for the first and sixth month.

Craft beers showed an aromatic profile much more intense than the commercial beers and kept the profile constant over the six months (as the commercial beers). The results allowed to conclude that the craft beers maintain the quality of a commercial beer, over six months, with the benefit of having most intense flavors and aromas.

Through the analysis of major compounds, no clear trends for ethanol and sugars concentrations were obtained. The concentration of organic acids on craft beers was higher than the concentrations typically found in commercial beers.

The results of minor compounds analysis were in line with the aromatic profiles obtained by sensory analysis, as well as those portrayed in the literature. The results showed that most of the principal aging markers reported were not found in the beers studied. However other compounds were found like higher alcohols, ketones and acids.

The validation of the method of extraction of minor compounds (used to analysis by gas chromatography–mass spectrometry) was conducted. To validate the method several parameters were studied: linearity, sensitivity, detection and quantification limits, precision (repeatability and intermediate precision), matrix effect, time effect and accuracy (spiking test).

The results showed that the method satisfies the specifications determined for each validation parameter. This means that the validation of the extraction method was a success.

**Keywords:** craft beer, ageing, flavor stability, sensory assessment, method validation, minor volatiles.

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AOAC – Association of analytical communities

GC-MS – Gas chromatography–mass spectrometry

HPLC – High-performance liquid chromatography

LD – Detection limit

LQ – Quantification limit

NA – Not applicable

R – Correlation coefficient

RI – Refraction index

RPM –Rotation per minute

RSD – Relative standard deviation

TCA – The tricarboxylic acid

UV – Ultraviolet

# 1. INTRODUCTION

Beer can be described as a beverage containing alcohol, extract and carbon dioxide. Beer is prepared from barley malt, hops, brewing water, and top- or bottom- fermenting yeast (Esslinger and Narziss 2003).

According to data collected by the association Brewers of Europe, the Portuguese brewing sector presented in 2012 a total production of almost 800 million liters. Portugal was, in 2012, the fourth largest EU beer exporter, exporting more than 40% of its total production. Portuguese brewers involve about 60.500 jobs, representing € 1.024 million in market value, and a per capita consumption value of 49 liters.

All beer types evolved from the combination of and relationships among: ingredients, processing, packaging, marketing and culture. When varying these bases, we create variety and distinct beer styles (Papazian 2006). The existing beer styles can be grouped into two classes: the bottom-fermented beer, also designated as lager beer and the top-fermented beer also called ale beer (Burberg and Zarnkow 2009).

There are several quantitative variables that differentiate one beer from another and that help define beer styles. These variables are a foundation from which we can begin to define beer types (Papazian 2006). The appearance of beer on its perception and parameters as alcoholic content, nutritive value, color, clarity, the absence of haze, the formation and retention of a good head of foam, and the absence of gushing, they all contribute to the satisfaction. Nevertheless it is the flavor, the taste and the aroma that really determine the acceptability and drinkability of the beer (Briggs *et al.* 2004).

The predominant influences on beer types are usually derived from hop bitterness and aroma, malt components (before and after yeast metabolism), the use of specialized malts and adjuncts. Since each type of beer uses different ingredients and processes, the final flavor will be different (Lewis and Young 1995).

The point where maturation ends and deterioration begins is undoubtedly distinct from beer to beer and probably different for the final consumer. The off-flavor in one beer may be an essential character of another (Briggs *et al.* 2004).

## 1.1. Beer Production Process

### 1.1.1. Raw Materials

#### Water

Water is the main component of beer and its quality for brewing is often determined by legislation. It has to be potable, pure, and free of pathogens, as measured by chemical and microbial analysis (Wunderlich and Back 2009).

#### Malt

Barley is the major source for brewing malts, which constitute the single most important raw material for beer production (Meussdoerffer and Zarnkow 2009). It does not have the necessary enzymes for brewing, it lacks friability for easy milling. The barley extract is very viscous, deficient in amino acids and it does not have the color and flavor required for making beer. The process of malting promotes all the modifications that are required in the physical, chemical and biological properties of barley. The result is malt which is the main raw-material for beer production (Lewis and Young 1995). Other crops like wheat, rye, triticale, spelt, and emmer are also suitable for brewing. Mostly they are added to barley malt (Wunderlich and Back 2009)

#### Adjuncts

Adjuncts are preparations of cereals (e.g., flaked maize or rice flakes, wheat flour, micronized wheat grains, or rice or maize grits which were cooked separately in the brewery) which may be mixed with ground malt in the mashing process (Briggs *et al.* 2004).

Adjuncts are usually considered non-malt sources of fermentable sugars and they typically contribute with no enzyme activity and little or no soluble nitrogen, being less expensive than malt (Stewart 2006a). The use of an adjunct alters the character of the beer produced (Briggs *et al.* 2004).

#### Hops

The common hop (*Humulus lupulus*) belongs within the botanical classification of urticales (nettle family), in the hemp family (*Cannabaceae*) (Roberts and Wilson 2006).

Hop gives beer its typical bitterness and aroma. Traditionally, it is added during brewing because of its preserving effects. Furthermore, hop contains pharmacologically active substances, as it is said to be soporific (Wunderlich and Back 2009). The antioxidant capacity of hop polyphenols is probably one of the main reasons for the health-promoting properties attributed to beer. Interest in employing antioxidants from natural sources to increase the shelf life of foods is

considerably enhanced by consumer preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants (Proestos and Komaitis 2009). Hop prevents the deterioration of the beers being very important for craft beers.

### 1.1.2. MALTING

The malting process consists in five stages: preparation of barley and its storage, steeping, germination, kilning, and preparation of the malt and its storage (Fig. 1) (Lewis and Young 1995).

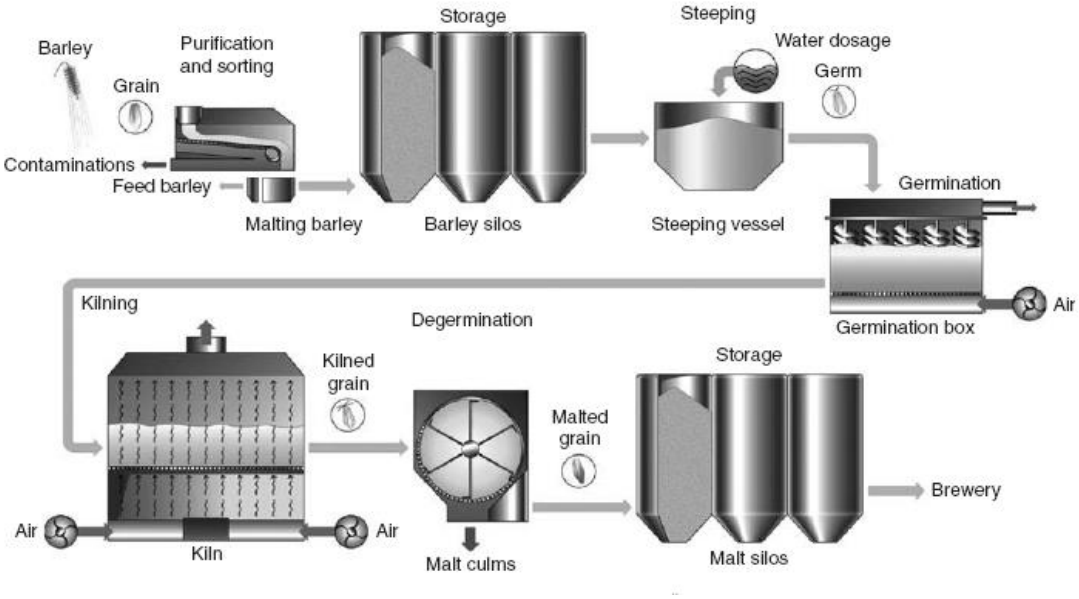


Fig. 1 - Schematics of the malting process. Source: Wunderlich and Back (2009).

For the preparation of barley and its storage, materials like stones, straw, leaves and twigs, pieces of metal, clods and dust or any strange material are removed. After cleaning, the grain is separated and graded (Lewis and Young 1995).

Steeping describes the stage in the malting process in which the grains, usually of barley or another cereal, are immersed or sprayed with water (Boulton 2013). Initially, the water uptake occurs as an osmotic process and depends on water quality and temperature, allowing the grains to respire (Esslinger and Narziss 2003). Moisture increases in steeping from about 10-12% to 42-46% and occasionally might reach higher values for special purposes (Lewis and Young 1995).

Germination is a physiological process where the embryo develops rootlets and acrospires. The aim of controlled germination is to produce a green malt with a defined composition, but not to allow the development of a new plant (Esslinger and Narziss 2003).

The green malt is dried by kilning in order to stop the chemical and biological transformations that takes place during germination. Kilning removes water, fixes substantial translations, and yields a product. Another function of kilning is to remove the vegetable-like flavor of the green malt and to impart to the kilned malt a specific aroma and a defined color characteristic for the type of malt required (Esslinger and Narziss 2003) (Wunderlich and Back 2009). At this stage, the moisture content of malt is reduced from about 45-50% to 1.5-5% with a current of heated air during 18 hours (Lewis and Young 1995, Esslinger and Narziss 2003) . An amount of 100 kg barley results in about 160 kg green malt and about 80 kg cured malt after drying (Wunderlich and Back 2009).

### 1.1.3. Wort Production and Biochemistry

The various stages of the brewing process are represented in Fig.2 and Fig. 3 and described in the follow subchapter.

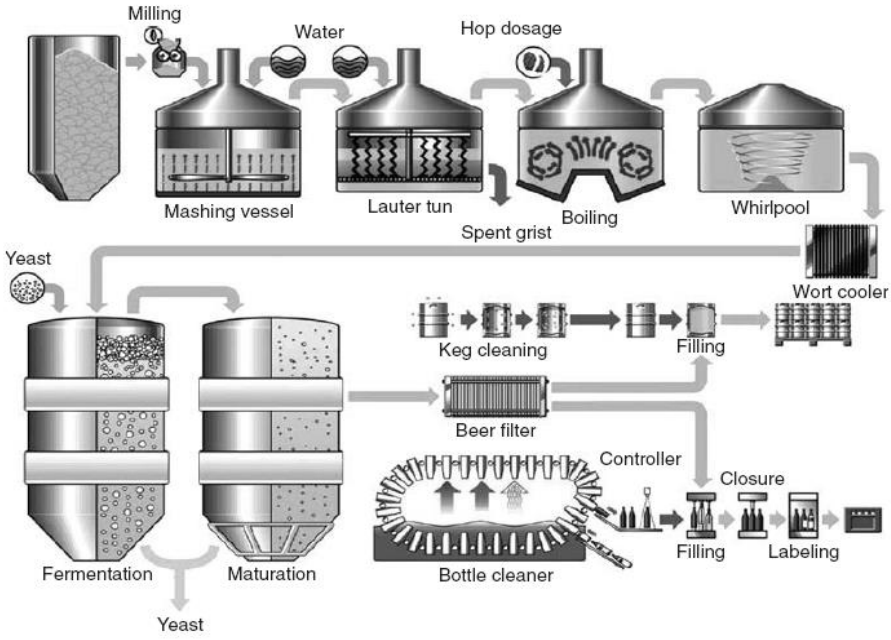


Fig. 2 - Brewing process schematically. Source: Wunderlich and Back (2009).

#### Milling

In this stage malt grains and other solid adjuncts are subjected to treatment - materials are broken and reduced in size to smaller particles, so that the best yield of extract may be easily obtained with the selected mashing system. The product that derives from milling is termed the grist (Boulton 2013). Here, the goal is to remove the husk exposing the starch, if the milling is to intense may form flour which would make difficult to filter the wort in further processing.

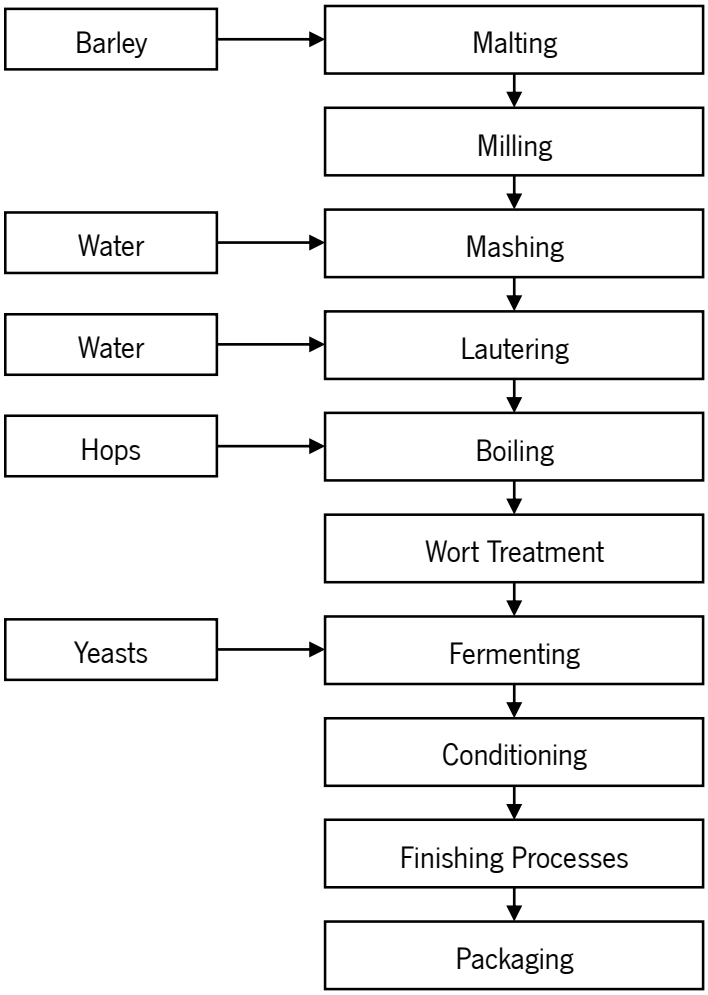


Fig. 3 – Brewing flowchart.

MASHING

This is the process in which malt grist, solid adjuncts, and water are mixed at a suitable temperature for the malt enzymes to convert the various cereal components into fermentable sugars and other nutrients. The liquid containing the nutrients is called wort or extract (Leiper and Miedl 2006). Starch breakdown is the main goal during this stage. Solubilization of starch granules proceeds in various steps. After swelling of the starch kernels, gelatinization of the starch occurs as enzymatic hydrolysis starts. Starch hydrolysis continues until no more $\alpha$-glucans (dextrins) are available. Protein hydrolysis is as important as starch hydrolysis, even though smaller amounts are transformed (Esslinger and Narziss 2003).

Enzymatic breakdown during mashing can be controlled manipulating temperature, viscosity, pH value and time.

LAUTERING

Lautering is a solid – liquid separation and aims to separate the compounds of malt dissolved during mashing from the insoluble parts. Usually, the systems used are the lauter tun and mash

filter (Krottenthaler *et al.* 2009). Those devices mainly differ in the independence from the quality of the malt and from the proportion of adjunct; quicker lautering of the more highly concentrated first wort; higher yields; mostly a hazier filtrate (Esslinger and Narziss 2003).

### Wort Boiling

The wort is transferred to a boiling device (kettle). According to the Manual of Good ± Wort Boiling and Clarification (Briggs *et al.* 2004) the following changes occur: inactivation of malt enzymes and sterilization of the wort; extraction and isomerization of compounds derived from hops; coagulation of protein material in the wort; formation of protein/polyphenol complexes, flavor and color complexes, reducing substances to give the wort reducing potential; fall in wort pH; concentration of wort gravity through evaporation of water and evaporation of volatile compounds in wort derived from mashing and volatile compounds in wort derived from hops.

Hop is added during wort boiling. That can be done at the beginning or end of boiling, or hop may be dosed into the whirlpool. Hop dosage at the beginning of wort boiling serves for enhancing and is generally carried out with bitter hop and a second dosage at the end of boiling or into the whirlpool gives a favorable hop dose (Wunderlich and Back 2009).

### Wort Treatment

Hot trub (hop particles and precipitated proteins) must be removed after boiling, or else the beer will taste wort-like, bitter, and even harsh. Typically a whirlpool separates the hot trub that settles down in the middle by the resulting rotation.

The wort must to be cooled down as fast as possible to minimize infection risk. It is cooled to 4–7°C for cold bottom fermentation, to 10–15°C for accelerated bottom fermentation, and to 12–18°C for top fermentation.

Then, proteins precipitate in wort again at temperatures below 60°C. Particles of this “cold trub” are smaller than those of hot trub. They are then removed by separation, filtration, or flotation (aeration of wort).

The oxygen necessary for yeast propagation (7–8 mg/L, corresponding to 80% saturation of the wort with $O_2$) is usually introduced in the form of air at the pitching temperature. If pure oxygen is used, it must be added carefully, not exceeding 15 mg/L since a higher level is detrimental to the yeast (Esslinger and Narziss 2003, Wunderlich and Back 2009).

### 1.1.4. FERMENTATION AND MATURATION

#### FERMENTATION

The main goal of fermentation is to convert sugars into ethanol and carbon dioxide as the major products of yeast metabolism. A series of minor metabolites such as esters, higher alcohols and acids that contribute positively to flavor are also produced by yeasts (Briggs *et al.* 2004).

Fermentation is initiated with the addition of 0.5 – 0.7 L of heavy yeast slurry per hectoliter of wort, corresponding to 15 – 20 million yeast cells per milliliter of cold and aerated wort. This stage is called the inoculation, pitching or seeding step. After the addition of yeast, the wort is called young beer or simply beer (Esslinger 2009).

The main consideration in primary fermentation is to ferment the wort to the desired gravity (degree of attenuation) in the required period of time (Lewis and Young 1995).

The temperature of fermentation for lagers, produced by bottom-fermenting yeasts, is usually in the range of 6–15 °C and takes 2–7 days, whereas ales are traditionally produced using top-fermenting yeasts and incubation temperatures of 18-27 °C for 5–7 days.

During fermentation of lager beer, the yeasts tend to flocculate and settle to the bottom of the fermentation vat, allowing collection and reuse in subsequent fermentations. By varying the fermentation temperature, slightly different versions of lagers can be produced. During fermentation of ale beer, yeasts tend to form small clumps of cells that are carried to the top of the fermenting liquid and adsorbed to bubbles of carbon dioxide. These yeast cells can be collected from the surface for reuse with the next fermentation batch.

Regardless of the type of beer made, a rapid decrease in pH during the fermentation will increase its stability and decrease potential problems of contamination. After fermentation, the pH of most lagers decreases from approximately 5.2–5.3 to approximately 4.1–4.2, and it decreases slightly more in ales. These acidic pH values assist in preserving the final product by inhibiting bacterial growth (Munroe 2006, Harrison 2009).

#### MATURATION

Beer, at the completion of primary fermentation is said to be ‘green'. It contains little entrained carbon dioxide, it is hazy and its taste and aroma are inferior to beer that is ready for sale. In order to refine green beer it must be matured or conditioned (Briggs *et al.* 2004). Traditionally, maturation involves a secondary fermentation that is effected by the small amount of yeast remaining beer when it is transferred from the fermenting vessel (Esslinger 2009).

Volatile substances like aldehydes and sulfur compounds are carried by $CO_2$ bubbles. Degradation of alpha-aceto-lactate and especially diacetyl takes place. Sedimentation of yeast clarifies the brew. Degradation of diacetyl as far as possible (<0.1 mg/l) fixes the end of maturation (Briggs *et al.* 2004, Wunderlich and Back 2009).

### 1.1.5. Finishing Processes

#### Clarification and filtration

Clarification of beer aims for the removal of yeast, sedimented protein and polyphenol haze material derived from the beer stabilization techniques and cold break. It is important to consider that the beer flavor is considerably more stable when it contains suspended yeast, as it promotes strongly reducing conditions. Total removal of yeast should therefore be delayed to the last possible moment before packaging.

Filtration generally refers to clarification of beer through several stages to produce a crystal-clear product. This step fulfills two roles: to remove suspended materials from the green beer and to unhinge potential turbidity formers. (Lindemann 2009).

#### Packaging

For filling beer, there are four main categories of packaging in use worldwide like glass bottles; cans made of aluminum or tinplate; plastic bottles or kegs.

The product is expected to be uniform with a long shelf life, rather than being instantly consumed before it can deteriorate. It is, therefore, vital that beer quality is maintained throughout its storage, which has become a huge technical challenge. Some factors are important in the choice of material to be used in packaging such as: light-proofing characteristics, barrier properties for preventing the escape of $CO_2$ or the entry of oxygen, inertness in terms of mass transfer between the packaging material and the product, and ability to withstand mechanical stresses and breakage (Alexander 2006, Blüml 2009).

#### Sterilization

To increase the shelf life of canned or bottled beer, it is usually pasteurized (1 min at 70°C) (Harrison 2009). The objective of pasteurization is to eliminate the possibility of undesired microbial contamination of beer or reduce it to the required low level. This depends on many factors including the kind of beer, the number and the species of microbes present and the size of the package (Lewis and Young 1995).

### 1.1.6. BREWING CONTAMINANTS

Beer becomes resistant to microbial contamination due to processes such as filtration, storage at low temperatures and pasteurization. The presence of inhibitors (such as hop compounds, alcohol, carbon dioxide and sulphur dioxide), the shortage of nutrients and oxygen and the low pH are conditions that also discourage the growth of microorganisms. The special environment in the brewing process restricts the range of microorganisms likely to be encountered to relatively few species. Although the contaminants found may cause quality defects (Table 1), pathogens have not been reported to grow in standard beer products (Storgårds 2000).

The range of microbes found in brewing processes is small, with three broad groups occurring: Gram-positive bacteria, Gram-negative bacteria and wild yeast (Lewis and Young 1995).

GRAM-POSITIVE BACTERIA

The main members of this group include lactic acid bacteria, *Leuconostoc, Streptococcus, Micrococcus* and *Bacillus.*

Lactic acid bacteria are the only group of Gram-positive bacteria likely to cause a significant threat to beer. *Lactobacillus* and *Pediococcus* are the two genera that are mostly encountered as potent beer-spoilage microbes (Lewis and Young 1995, Priest 2006).

GRAM-NEGATIVE BACTERIA

The main members of this group found in beer include Entereobactericeae, acetic acid bacteria, *Zymomonas, Pectinatus* and *Megasphaera* (Lewis and Young 1995).

WILD YEAST

By definition, wild yeast are any yeast other than the brewing strain(s) which is found in the brewing process. It is usual to consider two general types of wild yeast: those belonging to different genus to brewing yeasts (wild non-*Saccharomyces*) and those belonging to the genus *Saccharomyces* (Lewis and Young 1995).

Most wild yeasts can cause serious flavor effects and generally these yeasts are competing with the culture yeast for nutrients, but some yeast possesses the “killer” phenotype and actively kill sensitive culture yeast. These strains produce zymocins, proteins that are lethal to sensitive cells. Such killer strains can rapidly displace culture yeasts (Priest 2006).

In Table 1 are described different types of contaminants in the brewing process, as well as its effects in the final product like turidity or ropiness (increased viscosity).

Table 1 - Effects of contaminants during fermentation and on final beer. Adapted from Storgårds (2000)

| Group or genera | Effects on fermentation | Turbidity | Ropiness | Off-flavors in final beer |
|---|---|---|---|---|
| Wild yeasts | Super attenuation | + | - | Esters, fusel alcohols, diacetyl, phenolic compounds, H2S |
| *Lactobacillus, Pediococcus* | | + | + | Lactic and acetic acids, diacetyl, acetoin |
| *Acetobacter, Gluconobacter* | | +[1)] | +[1)] | Acetic acid |
| Enterobacteria | Decreased fermentation rate, formation of ATNC | - | - | DMS, acetaldehyde, fusel alcohols, VDK, acetic acid, phenolic compounds |
| *Zymomonas* | | +[2)] | - | H2S, acetaldehyde |
| *Pectinatus* | | + | - | H2S, methyl mercaptane, propionic, acetic, lactic and succinic acids, acetoin |
| *Megasphaera* | | + | - | H2S, butyric, valeric, caproic and acetic acids, acetoin |
| *Selenomonas* | | + | - | Acetic, lactic and propionic acids |
| *Zymophilus* | | +[3)] | - | Acetic and propionic acids |
| *Brevibacillus* | | - | + | - |
| *Clostridium* | | - | - | Butyric, caproic, propionic, and valeric acids |

ATNC; apparent total n-nitroso compounds, DMS; dimethyl sulphide, VDK; vicinal diketones, Fusel alcohols; n-propanol, iso-butanol, iso-pentanol, iso-amylalcohol

[1)] in the presence of oxygen, [2)] in primed beer, [3)] at elevated pH (5–6)

### 1.1.7. Beer Styles

All beer types evolve from the combination and relationship between ingredients, processing packaging, marketing and culture creating variety and distinct styles.

Lager Beers

In many countries, bottom-fermented beer is designated as lager beer. Its extract of original wort varies according to the local laws (tax classification) from 7 to 14%.

Bottom-fermented beer with an extract of original wort of 10 – 14% comprises an extraordinarily large variety of beer types, including pale and dark beers, export beers (more than 12% extract of original wort), Märzen beers, special beers and festival beers (13–14% extract of original wort). Within these limits there are such different beer types as Pilsner, Dortmunder, Munich, as well as smoky-flavor beer and cellar beers; these are, however, restricted to certain localities (Table 2) (Papazian 2006, Esslinger 2009).

Table 2 - Types of lager beers. Adapted from Papazian (2006)

| Origin | Types | | | |
|---|---|---|---|---|
| European Germanic | German Style Pilsner | Bohemian Style Pilsner | European Style Pilsner | Traditional German-Style Bock |
| North American | American Lager | American-Style Light Lager | American-Style Low-Carbohydrate Light Lager | American-Style Premium Lager |
| Other | Australasian, Latin American, or Tropical-Style Light Lager | Flavored Malt-Fermented Beverages | Flavored Malt-Fermented Beverages | Herb and Spice Beers |

ALE BEERS

Ale beers are top-fermented beers, which differ themselves in color and hop enhancement creating several types (Table 3). These beers differ from bottom-fermented beers in their ingredients (more than 50% wheat malt or other malted cereals) and by their special aroma, which is primarily induced by the top-fermenting yeast strains of *S. cerevisiae*. The particular yeast strain employed has a higher optimum fermentation temperature, and therefore the fermentation proceeds between 15 and 24°C. During fermentation, the yeast rises and can be skimmed off the top. At the higher fermentation temperatures, the diacetyl is easily decreased (Burberg and Zarnkow 2009, Esslinger 2009).

Table 3 - Types of ale beers. Adapted from Papazian (2006)

| Origin | Types | | | | |
|---|---|---|---|---|---|
| British | Classic English-Style Pale Ale<br>Scottish-Style Heavy Ale | English-Style India Pale Ale<br>Scottish-Style Export Ale | Ordinary Bitter<br>English- Style Dark Mild Ale | Special Bitter or Best Bitter<br>English-Style Brown Ale | Scottish -Style Light Ale<br>Imperial Stout |
| North American | American-Style Pale Ale | American-Style India Pale Ale | American-Style Amber／Red Ale | Golden or Blonde Ale | American-Style Stout |
| Belgian | Belgian-Style Flanders／Oud Bruin or Oud Red Ales | Belgian-Style Dubbel | South German-Style Dunkel Weizen／Dunkel Weissbier | Belgian-Style White (or Wit)／Belgian - Style Wheat | Belgian-Style Lambic |
| German | German-Style Brown Ale／Düsseldorf - Style Altbier | South German-Style Hefeweizen ／Hefeweissbier | South German-Style Kristal Weizen／Kristal Weissbier | Belgian-Style Tripel | |
| Irish | Irish-Style Red Ale | Classic Irish-Style Dry Stout | | | |

## Craft beer

According to the Brewers Association (2014) a craft brewer is small, independent and traditional. The following concepts are related to craft beer and craft brewers:

- Craft brewers are small brewers;
- The hallmark of craft beer and craft brewers is innovation;
- Craft beer is generally made with traditional ingredients like malted barley; interesting and sometimes non-traditional ingredients are often added for distinctiveness;
- Craft brewers tend to be very involved in their communities through philanthropy, product donations, volunteerism, and sponsorship of events;
- Craft brewers have distinctive, individualistic approaches to connecting with their customers;
- Craft brewers maintain integrity by what they brew and their general independence, free from a substantial interest by a non-craft brewer.

# 2. FERMENTATION BIOCHEMISTRY

## 2.1. YEAST

Yeast is the leading organism during alcoholic fermentation, this section will focus on yeast taxonomy, nutritional requirements and metabolism.

### 2.1.1. TAXONOMY

The yeast is the most important microorganism for producing fermented beverages like beer (Lewis and Young 1995). The interest in brewing yeast centers on the existence of thousands of unique strains of *Saccharomyces cerevisiae.*

*Saccharomyces*, Latin for sugar fungus, is the name first used for yeast in 1838 by Meyen, but it was the work of Hansen at the Carlsberg laboratory in Denmark during the 1880s that gave us the species names of *S. cerevisiae* for top-fermenting yeast used in ale fermentations and *S. carlsbergensis* for bottom-fermenting yeast associated with the lower temperature range of lager fermentations.

The taxonomy surrounding the yeast *Saccharomyces* is confusing and still changing. *Saccharomyces sensu stricto* is a species complex that includes most of the yeast strains relevant in the fermentation industry as well as in basic science (i.e., *S. bayanus*, *S. cerevisiae, S. paradoxus*, and *S. pastorianus*) (Russell 2006).

### 2.1.2. NUTRITIONAL REQUIREMENTS

Some chemical components present in the wort or other medium surrounding yeast cells may be used as nutrients, some may be toxic or growth-suppressing and others may have no effect whatsoever. In some cases, the same component may be a nutrient at a low concentration or toxic at a higher concentration. Some substances are assimilated only under particular growth conditions unlike the major classes of nutrients such as sources of carbon and nitrogen which are assimilated in an ordered fashion. Thus, where several sources of carbon and nitrogen are present, the yeast first utilizes those which are most readily assimilated (Briggs *et al.* 2004). Brewer's yeast needs available sources of carbon, nitrogen, certain vitamins, trace elements and under normal circumstances a small amount of molecular oxygen (Lewis and Young 1995):

#### OXYGEN REQUIREMENTS

Under brewing conditions, oxygen can be considered as a nutrient for yeast. Although yeast goes through long anaerobic phases during fermentation and most of the respiratory pathways are

blocked due to the Crabtree effect, yeast needs oxygen for sufficient growth (Tenge 2009). This requirement is justified because brewing yeasts need molecular oxygen to synthesize sterols and unsaturated fatty acids that are present in wort at suboptimal concentrations (Russell 2006).

### Carbohydrates and Fermentable Sugars

Regular wort contains mainly the following sugars: fructose, glucose, sucrose, maltose, maltotriose and dextrins. All sugars can be utilized for the generation of energy and biosynthesis, except for the dextrins. These fermentable sugars are the main carbon source for the yeast. After being pitched, yeast immediately ingests the monosaccharides. Another important carbohydrate source for yeast is glycogen. It serves as a reserve carbohydrate and is generated in the cell during the later anaerobic stages of fermentation (Tenge 2009).

### Nitrogen Sources

Yeasts cannot assimilate gaseous nitrogen, however simple inorganic sources such as ammonium salts may be readily utilized. In natural media, such as brewers' wort, ammonium ions, amino acids, peptides, purines and pyrimidines provide most of the nitrogen needed. Many of these are used as a source of nitrogen only in the presence of additional sources of carbon and energy (Briggs *et al.* 2004).

### Minerals and Trace Elements

Metals are very important for yeast cell physiology. They are needed to maintain the cell's structural integrity, flocculation, gene expression, cell division, nutrient intake, enzyme activity, etc. The most important metals that influence yeast fermentation are potassium, magnesium, calcium, manganese, iron, copper, and zinc (Tenge 2009).

Brewers' malt wort supplies all the mineral nutritional requirements of yeast, with the possible exception of zinc. For this reason, zinc supplements are commonly added to wort in the fermenter (Briggs *et al.* 2004).

### Vitamins and Other Growth Factors

This group contains mainly organic compounds that are needed in very low concentrations. For yeast, these are purines, pyrimidines, fatty acids and vitamins that are used as components of cofactors. Due to the low amounts in which the yeast needs these substances, they do not play a critical role in regularly prepared worts (Tenge 2009).

### 2.1.3. METABOLISM

Metabolism is the sum of all the chemical processes occurring in a cell. The manifestations of metabolism are the disappearance of nutrients from the medium and the appearance of by-products, the growth of individual cells and cell proliferation (Briggs *et al.* 2004).

#### METABOLISM OF WORT SUGARS

The dominating metabolic pathway of brewer's yeast during beer production is the formation of ethanol by consumption of wort carbohydrates. In general, alcoholic fermentation is an energy generation under anaerobic conditions, where glucose is metabolized to ethanol and $CO_2$ (Tenge 2009).

Glycolysis, or the Embden-Myerhof-Parnas pathway (Fig. 4), is the major sugar catabolic pathway in yeast and it operates under both aerobic and anaerobic conditions and is the route by which approximately 70% of exogenous hexose sugars are assimilated.

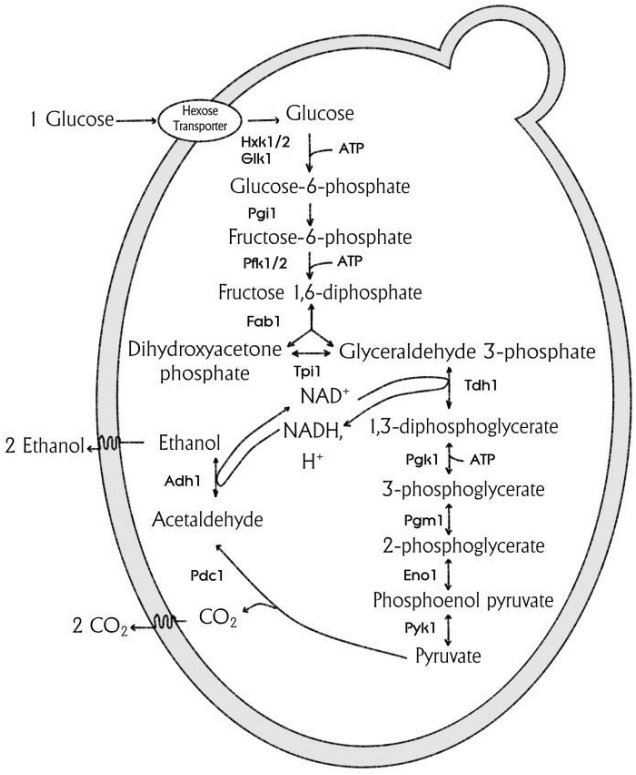


Fig. 4 - Alcoholic fermentation: enzymatic steps on *S. cerevisiae*. Source: Faria-Oliveira *et al.* (2013).

In oxidative metabolism, some of the pyruvate derived from glycolysis is oxidized to acetyl coenzyme A, which is then oxidized to two molecules of carbon dioxide in a series of reactions variously termed the citric acid cycle, tricarboxylic acid cycle (TCA) or Krebs cycle (Fig. 5).

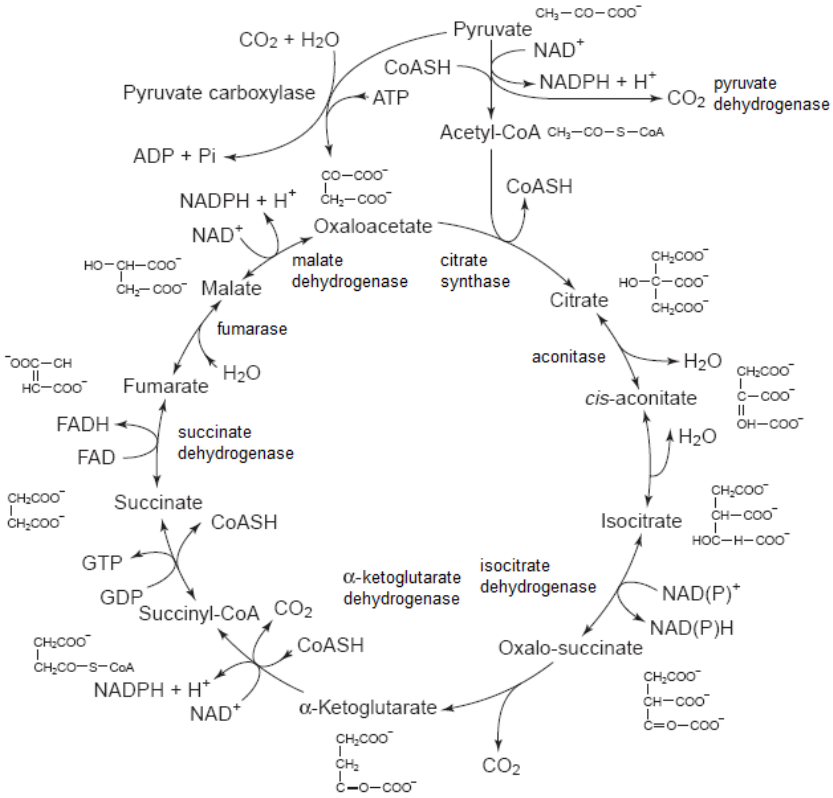


Fig. 5 - The tricarboxylic acid (TCA) cycle. Adapted from Briggs *et al.* (2004).

### METABOLISM OF AMINO ACIDS

Wort amino acids are metabolized in two ways: they may be taken into the cell and incorporated directly into proteins, or the amino group may be transferred to an enzyme (transaminase) and the remaining carbon skeleton secreted or used to regenerate $NAD^+$. The compounds formed by the amino acids metabolism like higher alcohols and aldehydes influence the beer flavor (Lewis and Young 1995).

### METABOLISM OF LIPIDS

The biosynthesis of new yeast cells during fermentation requires the synthesis of lipids. They have important structural roles, especially in membranes. The synthesis of fats and sterols essentially begins with acetyl coenzyme A to produce saturated and unsaturated acetyl CoA molecules and sterols. Biosynthesis of unsaturated molecules and sterols requires and oxidative step. Oxygen deficiency results in poor fermentation and in an increased level of acetyl CoA in the cell, which can lead to elevated levels of esters in the beer, influencing flavor (Lewis and Young 1995, Briggs *et al.* 2004).

## 2.2. KEY COMPOUNDS FOR IMPROVED FLAVOR AND AROMA

The principal flavor metabolites are aliphatic alcohols, aldehydes, organic and fatty acids and esters. These are formed as by-products of the metabolism of sugars and amino acids (Fig. 6). Many of these are excreted by yeast during fermentation. However, some are intracellular components that are released in the beer either by cell death and autolysis or via shock excretion (Briggs *et al.* 2004).

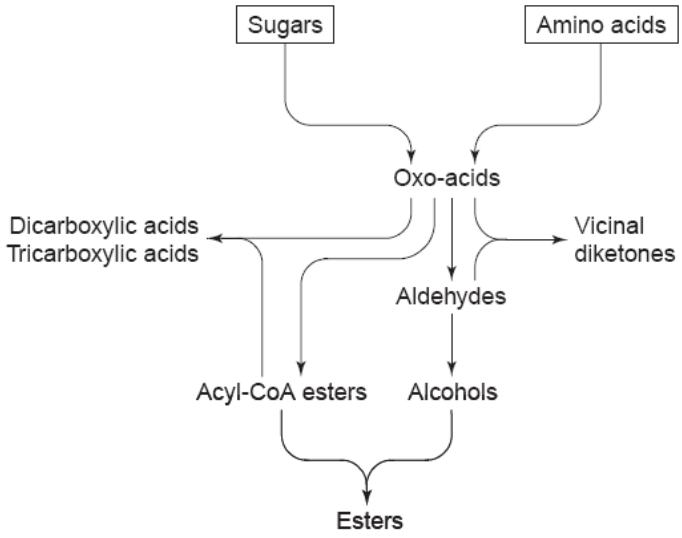


Fig. 6 - Relationships between the major classes of yeast-derived beer flavor compounds. Source: Briggs *et al.* (2004).

Fermentation conditions and nutritional supplements are important in beer brewing due to their influence on fermentation performance and final product characteristics (Hiralal *et al.* 2014).

### 2.2.1. ALCOHOLS

In addition to ethanol, several other alcohols are found in beer, such as higher alcohols or fusel oils which contribute significantly to flavor (like *n*-propanol, iso-butanol, iso-amylalcohol, 2-methylbutanol, phenylethanol and tyrosol).

Higher alcohol formation is related to yeast protein synthesis. They can be synthesized via two routes as by-products of amino acid assimilation (the catabolic route) or *de novo* from wort carbohydrates (the anabolic route).

These higher alcohols represent the majority of the volatiles in beer. The flavor impressions reach from flowery to solvent-like and alcoholic (Russell 2006, Tenge 2009).

### 2.2.2. ESTERS

During fermentation, the most important group of flavor-active compounds that are formed by yeast is the esters. More than 100 esters have been detected in beers. Esters have fruity/solvent-like aromas and flavors (Table 4) (Briggs *et al.* 2004).

They are formed intracellularly by an enzyme-catalyzed condensation reaction between two co-substrates, a higher alcohol and an activated acetyl-coenzyme A (acetyl-CoA) molecule (Russell 2006). Since the main alcohol in yeast is ethanol the most common ester is ethyl acetate (Lewis and Young 1995).

Table 4 – Examples of esters found in beer. Adapted from: Tenge (2009)

| Ester | Flavor impression |
|---|---|
| Ethyl acetate | fruity, like a solvent |
| Isobutyl acetate | fruity |
| Isoamyl acetate | fruity, banana |
| Ethyl butyrate | apple, papaya |
| Ethyl hexanoate | soapy, estery |
| Ethyl dodecanoate | fruity, strawberry |

### 2.2.3. CARBONYL COMPOUNDS

Carbonyl compounds are abundant in beers and more than 200 have been detected. The concentrations of several aldehydes and the vicinal diketones are influenced by yeast metabolism during fermentation and subsequent conditioning. As a group, these generally have a negative contribution to beer flavor and aroma (Lewis and Young 1995, Russell 2006). Therefore an important requirement of fermentation management is to ensure that these compounds are reduced to acceptable concentrations. Excessive concentrations of carbonyl compounds are known to cause a stale flavor in beer. The carbonyl found in highest concentration in beer is acetaldehyde (Briggs *et al.* 2004).

Diacetyl (butane-2,3-dione) and the related compound pentane-2,3-dione are produced from yeast metabolites which are secreted into beer (Lewis and Young 1995). These two compounds are of critical importance in the fermentation of lager beer (Briggs *et al.* 2004). Both vicinal diketones are formed from intermediates of the amino acid biosynthesis: diacetyl relates to valine and 2,3-pentandione relates to isoleucine (Tenge 2009).

### 2.2.4. ORGANIC AND FATTY ACIDS

More than 100 organic and fatty acids have been identified in beer. Although some of these are derived from wort, many are produced as a result of yeast metabolism.

Organic acid formation and excretion contributes to the reduction in pH that occurs during fermentation, being the most abundant organic acids found in beers: acetic, citric, lactic, malic, $\alpha$-ketoglutaric, pyruvic and succinic acids. They confer a 'sour' or 'salty' taste to beers.

Short and medium chain length fatty acids have unpleasant flavors and they inhibit beer foam formation so their presence in beer is undesirable. Generally, the medium chain-length fatty acids, principally $C_{16}$ and $C_{18}$, of wort are replaced by shorter chain-length fatty acids ($C_6$-$C_{10}$) in beer (Briggs *et al.* 2004).

### 2.2.5. SULFUR COMPOUNDS

Sulfur is of upmost importance in brewing because traces of volatile sulfur compounds such as hydrogen sulfide, dimethyl sulfide (DMS), sulfur dioxide and thiols contribute significantly to the flavor of the beer. DMS is an important beer flavor compound, derived from the wort production process and via yeast metabolism, being its flavor described as cooked sweet corn or cooked vegetable. At low levels, it is considered an essential flavor component, contributing to the distinctive flavor and aroma of lager beer, but at high concentrations it is objectionable (Russell 2006).

## 2.3. Flavor Stability

For beer flavor, stability means the ability to keep its characteristics unaltered from the time of filling to the time of consumption (Fig. 7) (Gresser 2009).

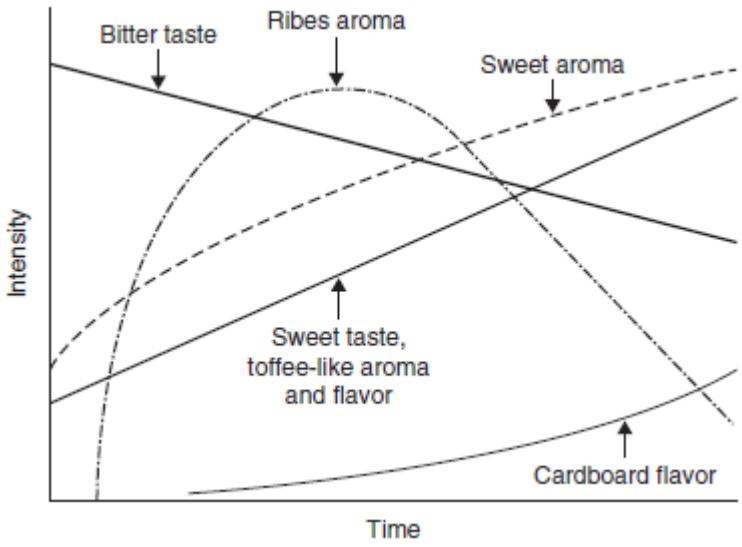


Fig. 7 - The Dalgliesh plot. Changes in various flavors in beer as the beer is stored over time. Source: Hernández and Milla (2009).

The staling process is characterized by oxidation reactions of natural beer components (higher alcohols, melanoidins, amino acids, fatty acids, hop resins) and several components are formed. The changes in beer flavor during its conservation are divided into two groups: changes in freshness and in bitterness (less harmony in taste compared with the initial taste) and changes in aroma (appearance of lightstruck flavor) (Gresser 2009).

It is clear that beer flavor stability is influenced by all stages of the brewing process: preservation of reducing substances by avoidance of oxygen pick-up during mashing, lautering, and wort boiling; elimination of substances that are prone to react with flavor-active compounds such as carbonyl molecules by good mashing and wort separation procedures, and prevention of ion pick-up such as iron and copper. Controlled exposure of the wort to heat is important to limit the formation of Maillard reaction products and related substances. The role of such products in beer staling reactions is ambiguous, and there are reports of their positive and negative influences (Stewart 2006b).

### 2.3.1. REACTION MECHANISMS OF AGING PROCESSES IN BEER

Even if there is no gustatory perception of changes in beer flavor, chemical composition of beer is changing. It is impossible to refer only one mechanism or a limited series of mechanisms identifying the processes inducing the degradation of the beer aroma. Several mechanisms lead to the formation of carbonyl compounds. The simple scheme shown in Fig. 8 shows the degradation processes (Gresser 2009).

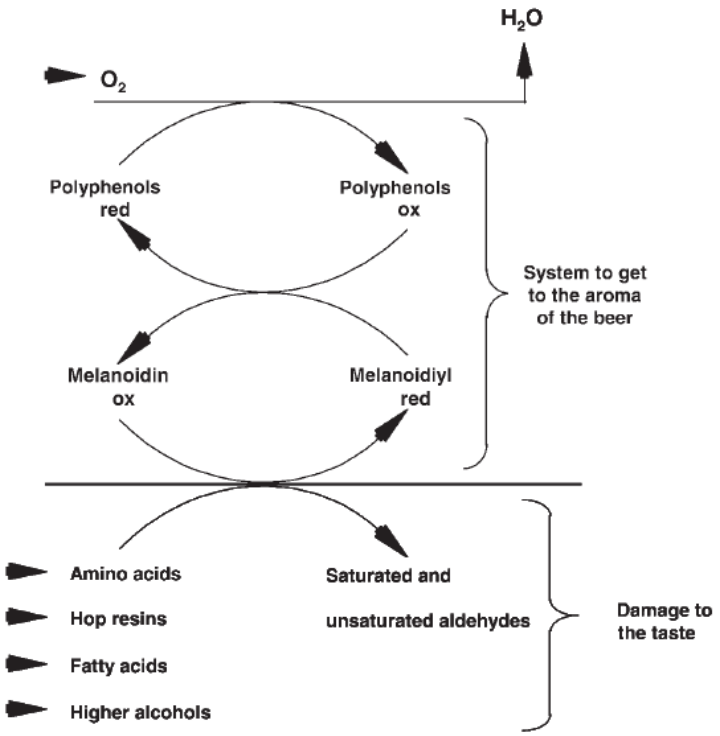


Fig. 8 - Redox reactions that promote the formation of staling flavor. Source: Gresser (2009).

#### 2.3.1.1. AGING REACTIONS PRODUCING CARBONYL COMPOUNDS

During aging numerous reactions are important, where carbonyl formation can be enhanced by the following factors:

- Strecker's degradation of amino acids

The reaction involves transamination, followed by decarboxylation of the subsequent α-ketoacid, resulting in an aldehyde with one carbon atom less than the amino acid. Strecker aldehydes have potent aromas and tastes which are generally unpleasant at high concentration (Vanderhaegen *et al.* 2006).

- Oxidations of higher alcohols

The most important alcohols in beer are ethanol, 2-methyl-propanol, 2-methyl-butanol, 3-methyl-butanol and 2-phenyl-ethanol. The concentrations of the corresponding aldehydes increase during beer aging, in particular when oxygen is present (Vanderhaegen *et al.* 2006).

- Aldol condensation of aldehydes

In these reactions, amino acids may be the basic catalysts through the formation of an imine intermediate. This pathway can produce carbonyl compounds with lower flavor thresholds from carbonyls present in beer which are less flavor active, which can be formed by other pathways (Vanderhaegen *et al.* 2006).

- Degradation of hop bitter acids

The degradation of hop bitter acids not only decreases sensory bitterness, but also results in the formation of by-products. For example, the degradation of the carbonyl side-chain of $\alpha$-acids and $\beta$-acids releases 2-methyl-propionic acid, 2-methyl-butyric acid and 3-methyl-butyric acid lowering the overall bitterness and the freshness and herbal character of hoppy beers (Vanderhaegen *et al.* 2006).

- Oxidation of unsaturated fatty acids

There are two pathways for the oxidation of unsaturated fatty acids: enzymatic oxidation and autoxidation. Despite all the beer-aging models, actual lipid oxidation does not seem to occur in bottled beer at normal storage temperatures. The total amount of (E)-2-nonenal in aged beer originates from autoxidation during wort boiling and enzymatic action during mashing (De Schutter *et al.* 2009).

- Formation of (E)-$\beta$-damascenone

(E)-$\beta$-damascenone belongs to a class of carotenoid derived carbonyl compounds. Potential precursors of damascenone in beer are allene triols and acetylene diols formed by degradation of neoxanthin, which is present in the basic ingredients of beer (Gresser 2009) (Vanderhaegen *et al.* 2006). Damascenone can directly affect beer flavor during ageing, generating a pleasant stewed apple, fruity and honey-like character (Rodrigues and Almeida 2009).

#### 2.3.1.2. ACETALIZATION OF ALDEHYDES

A condensation reaction between 2,3-butanediol and an aldehyde (acetaldehyde, isobutanal, 3-methylbutanal and 2-methyl-butanal, respectively) originate the cyclic acetals (2,4,5-trimethyl-1,3-dioxolane, 2-isopropyl-4,5-dimethyl-1,3-dioxolane, 2-isobutyl-4,5-dimethyl-1,3-dioxolane and 2-sec butyl-4,5-dimethyl-1,3-dioxolane) (Vanderhaegen *et al.* 2006). 2,4,5-trimethyl-1,3-dioxolane will

increase similarly to the increase of acetaldehyde therefore this molecule may become a suitable marker for bottled beer oxidation during aging (Vanderhaegen *et al.* 2003, De Schutter *et al.* 2009).

#### 2.3.1.3. MAILLARD REACTION

Reaction between amines or amino acids and carbonyl compounds, especially reducing sugar (Briggs *et al.* 2004). The most important Maillard compounds are 2-furfural, 5-methyl-2-furfural, 2-acetylfuran, 2-furanmethanol and thiazole (Vanderhaegen *et al.* 2003).

The Maillard reactions are associated with browning reactions and the formation of flavored compounds, particularly toffee/caramel-type flavors and aromas. The colored compounds that arise from these reactions are termed melanoidins. Maillard reactions form the basis of many of the transformations that underpin color and flavor changes associated with the kilning stage of malting and the boiling stage of wort production. The combination of the reactions that occur during malting and wort boiling is responsible for the color of beer (Boulton 2013).

#### 2.3.1.4. SYNTHESIS AND HYDROLYSIS OF VOLATILE ESTERS

Chemical condensation reactions between ethanol and beer organic acids occur at significant rates during beer storage. For example, 3-methyl-butyric acid and 2-methyl-butyric lead to ethyl 3-methyl-butyrate and ethyl 2-methyl-butyrate. These compounds impart a winey, brandy-like flavor to beer (Vanderhaegen *et al.* 2006, De Schutter *et al.* 2009).

#### 2.3.1.5. OTHER REACTIONS

- Formation of dimethyltrisulfide (DMTS)

Reaction between methanesulfenic acid and hydrogen sulfide leads to DMTS formation during beer storage. Methanesulfenic acid is formed by β-elimination from S-methylcysteine sulfoxide, introduced to beer from hops.

- Degradation of polyphenols

Simple polyphenols polymerize to high molecular weight species (tannins), either by acid catalysis, or by oxidative mechanisms. During beer storage, phenolic polymers interact with proteins and form insoluble complexes and hazes (Vanderhaegen *et al.* 2006).

# 3. BEER FLAVOR AND SENSORY ASSESSMENT

## 3.1. FLAVOR

Perception of flavor involves the individual senses of touch, taste and smell. The sense of touch is used to perceive so-called mouth-feel characteristics (like smoothness, astringency, temperature and the tingling sensation). Taste is perceived by the taste buds of the tongue and our primary tastes are recognized – sweet, salt, sour and bitter (Fig. 9).

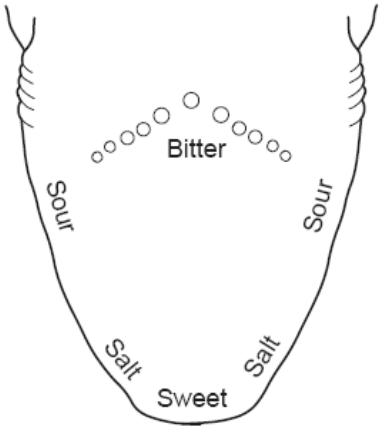


Fig. 9 - Areas of the human tongue where the four tastes are most easily sensed. Source: Briggs *et al.* (2004).

It is quite common for individuals to confuse the senses of smell and taste. The flavor of any beverage taken into the mouth is virtually simultaneously discerned by both senses. This happens as volatile compounds pass up into the olfactory organ at the back of the nasal cavity. Both the senses are influenced by physiological, psychological and genetic factors (Lewis and Young 1995).

## 3.2. FLAVOR ATTRIBUTES IN BEER

Taylor and Organ (2009) described some of the flavors commonly found in beers. As well as aroma and taste descriptions, some notes on the origin of the flavors were also described as shown in Table 5.

Table 5 - Aromas and taste descriptions. Adpted from: Taylor and Organ (2009)

| Descriptor | Aroma and Taste |
|---|---|
| Sulfur Dioxide | Pungent/choking aroma/sensation |
| Hydrogen Sulfide / Mercaptan | Rotten eggs. |
| Dimethylsulfide | Cooked sweet corn, cooked cabbage or processed tomatoes. |
| Solvent | Solvent-like flavor, nail varnish |
| Acetaldehyde | Green apples, raw apple skin or bruised apples. |
| Estery / Fruity | Banana, pear, apple, boiled lollies and aniseed |
| Hoppy | Taste attribute - aroma resulting from hop pellet or hop oil addition rather than the bitterness coming from hop pellets |
| Floral | Aroma of flowers, fragrant, rose-like and perfume-like |
| Spicy | Spicy flavor |
| Fresh Grass | Freshly cut grass like character of dried grass. |
| Clove/4 – VG | Clove-like flavor |
| Grainy/Straw | Aroma of barley, dried grass, straw or hay-like. |
| Malty | Flavor of malt or malt extract. |
| Caramelized | Caramel or toffee like. |
| Roasted | Roasted barley or malt, sugar, chocolate malt and smoky. |
| Fatty Acid | Soapy, waxy or sweaty. |
| Butyric | Vomit-like. |
| Cheesy | Cheesy flavor |
| Diacetyl | Butter, butterscotch or honeycomb. |
| Yeasty | Flavor of autolysed yeast or yeast extracts |
| Oxidized | Complex attribute as it covers a number of different flavors |
| Acidic/Sour | Taste sensation (aroma can arise - excessive levels of acetic acid) |
| Alcoholic | Alcoholic/warming effect |
| Body | Effect of the beer on the inside of the mouth, including the after - palate effect. |
| Sweetness | Taste sensation - taste of sucrose or honey. |
| Bitterness | Taste sensation |
| After - bitterness | Bitter taste that lingers after the sample has been swallowed |
| Astringency | Mouthfeel sensation characterized by 'mouth - puckering' as experienced when drinking strong black tea or young red wine. |
| Metallic | Taste sensation - rusty water and tinny. |

## 3.3. SENSORIAL EVALUATION

The options available for the sensory evaluation of beers depend on the question posed by the experimentalist. The circumstances in which sensory data will be required are diverse as, for example, ensuring acceptability of an established product prior to its release to the market or ensuring that any (minor) process change does not affect the flavor attributes of a brand, define the sensory attributes of unfamiliar brands (e.g. new own brands, competitor brands) or troubleshoot possible flavor defects.

Each of the above circumstances requires different skills of the taster and different questionnaires to be posed by the sensory analyst. (Hughes 2009):

### 3.3.1. TYPES OF SENSORY TESTS

Despite this fundamental limitation, brewers and flavor analysts have developed robust procedures that enable sensory analysis to be a valuable tool in the monitoring and control of beer quality. There are five basic types of flavor evaluation methods described by Philliskirk (2006):

DIFFERENCE TESTS

These tests are employed by trained tasters to establish if there is a difference between one or more samples.

In the triangular taste test: three beers are presented, two of which are identical and the third is from a different batch. Similarly, the duo–tri test also uses three samples but here a control beer is used as a reference and is tested against the same control beer and a test beer; the taster must match the reference. Difference tests are useful for monitoring consistency in a beer and if changes to the process or raw materials have affected beer quality.

DESCRIPTIVE TESTS

These tests rely on highly trained assessors to estimate the flavor of beers using an established vocabulary of flavor terms. These terms were agreed internationally during the 1970s to describe the characteristic flavors found in beer. Each flavor was given a specific name (Chapter 3.2.) and a chemical was assigned to each character to act as a standard reference. This vocabulary is characterized by the Beer Flavor Wheel (Fig. 10). Descriptive tests can be used in establishing the flavor profile of a beer or in trueness-to-type tests.

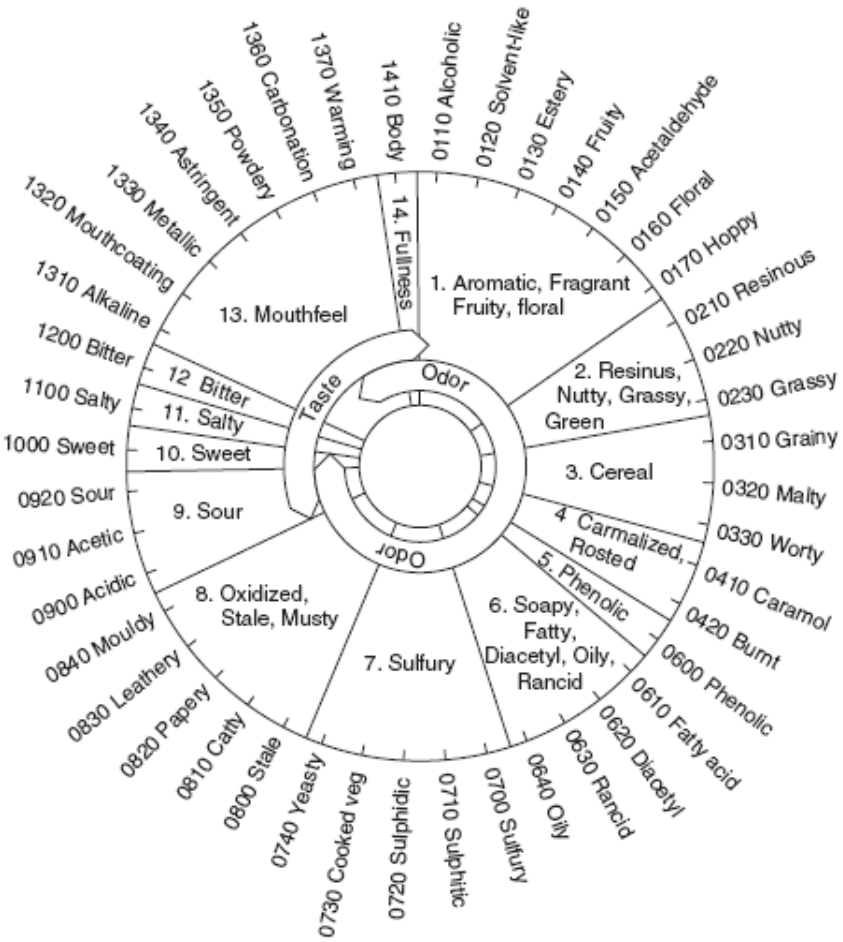


Fig. 10- The Meilgaard beer flavor wheel. Source: Hernández and Milla (2009).

PREFERENCE TESTS

The need to understand the likes and dislikes of consumers is critical. Preference tests, in which consumers are asked to compare two beers, express a preference, and sometimes comment on any perceived character ("too bitter," "too gassy"), are used to monitor a beer's performance in the market against its competitors.

SCALING TESTS

These tests are designed to quantify or rank particular flavor attributes or defects in a beer. Trained tasters are used.

DRINKABILITY TESTS

Most sensory analysis involves the smelling and drinking of relatively small quantities of beer. However, consumers will usually drink significantly greater volumes of beer and this can be used as a measure of acceptability or "drinkability" of a particular beer. In consumer tests where beers are being compared and tasted "blind" over a protracted session, the actual volume of beer consumed is a good indicator of the preference of the consumer.

### 3.3.2. TASTERS

The competence of the tasters determines the success or failure of each and every sensory test. Sometimes it seems as if this important point is not as obvious as it appears (Simpson 2006). Tasters are selected on the basis of their ability to discriminate between certain flavors and aromas. Not everyone can demonstrate the required level of sensitivity, and those that can, require training in identifying and naming the range of characteristics found in beer. This process can take several months before a taster qualifies for membership of the taste panel. Subsequently, taste panelists should be periodically exposed to standard flavors and smells to test acuity (Philliskirk 2006).

### 3.3.3. TRAINING PROGRAMME

In a brewery there are three applications that have wide use and provide a focus for training and panel selection. These are assessment of difference, beer quality acceptance assessment and beer flavor profiling. These three applications require different levels of training:

- Level 1 - the most basic level - the 'Difference Panel';
- Level 2 - a more complex level - the 'Beer Quality Acceptance Panel';
- Level 3 - involves a 'Profile Panel'.

The training program begins with a description of the technique for flavor assessment and then takes the panelists through the various stages of Level 1, Level 2 and Level 3. Larger numbers are more efficient but once a panel is established new recruits are usually introduced in smaller numbers (Taylor and Organ 2009).

### 3.3.4. SENSORY EVALUATION ENVIRONMENT

Tasting rooms should be specifically designed for the purpose, with the following basic conditions: be quiet, dim light, good ventilation and air conditioning, odor free (no smoking extraneous smells), separation of tasters, preferably in booths. The tasters must not use perfumes or consume food or drink immediately before the session (Philliskirk 2006).

# 4. ANALYTICAL METHODS

Besides the sensory analysis and in order to better understand the results sent by these, other tests can be done, including analytical methods.

## 4.1. HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPHY)

Beer constituents can be divided into volatile and non-volatile components. The non-volatile constituents include inorganic salts, sugars, amino acids, nucleotides, polyphenols and hop resins together with macromolecules such as polysaccharides, proteins and nucleic acids. Such compounds are usually resolved by high precision liquid chromatography (HPLC) (Briggs *et al.* 2004).

## 4.2. GC-MS (GAS CHROMATOGRAPHY MASS SPECTROMETRY)

The volatile components have greater vapor pressure and are responsible for the bouquet or aroma of beer. They are concentrated in the headspace above the liquid in a closed container and will pass into the distillate if the beverage is distilled. The complex mixture of volatile components either in the headspace or in a solvent extract of the beer can be resolved by gas-liquid chromatography, using either packed or capillary columns, and the components identified by mass spectrometry (GC-MS) (Briggs *et al.* 2004).

Unlike HPLC, which works by direct sample injection, the GC-MS requires an extraction for subsequent injection. In this work is proposed a fast and simple extraction method able to quantitate many of the most important volatiles, not only in beer but in other beverages too, in a single chromatographic run. However, as any other new method, this needs to be validated to ensure that every future measurement in routine analysis will be close enough to the unknown true value for the content of the analyte in the sample (González and Herrador 2007).

## 4.3. VALIDATION OF ANALYTICAL METHODS

"The word validation originates from the Latin *validus* meaning strong, and suggests that something has been proved to be true, useful and of an acceptable standard" (Araujo 2009) .

Methods should be validated or revalidated (Huber 1998) :

- before their introduction into routine use;

- whenever the conditions change for which the method has been validated, e.g., instrument with different characteristics;
- whenever the method is changed, outside the original scope of the method.

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to the unknown samples analyzed in the routine. The parameters for method validation have been defined in different working groups of national and international committees and are described in literature. Unfortunately some of the definitions are different between organizations (Huber 1998).

The parameters that should be analyzed depend on the purpose of the method, doesn't exist a specific protocol that defines the parameters that need to be evaluated. Those that were considered most important for this work were: linearity, sensibility, detection and quantification limits, precision and accuracy.

### 4.3.1. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly, or by means of well-defined mathematical transformations, proportional to the concentration of analytes in samples within a given range. Linearity is determined by a series of three to six injections of five or more standards whose concentrations cover the expected concentration range. The response should be directly or by means of a well-defined mathematical calculation proportional to the concentrations of the analytes (Huber 1998) .

### 4.3.2. Sensibility

If the calibration curve is defined by a linear model, the sensibility is constant over the entire working range and equal to the slope of this calibration curve. A method is said to be sensitive if small changes in concentration cause larger changes in the response function (Causon 1997).

### 4.3.3. Detection and Quantification Limits (LD) (LQ)

The detection limit of an analytical procedure is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated as an exact value.

The limit of quantification is the lowest amount of the analyte in the sample that can be quantitatively determined with defined precision under the stated experimental conditions.

The detection limits (LDs) and quantification limits (LQs) are calculated from the calibration curves as 3 (Eq. 1) and 10 (Eq. 2) times the ratio between the standard deviation of a response (s) and the slope of the analytical curve (b) (Ribani *et al.* 2007):

$$LD = 3.3 \times \frac{s}{b} \qquad \text{Eq. 1}$$

$$LQ = 10 \times \frac{s}{b} \qquad \text{Eq. 2}$$

### 4.3.4. PRECISION

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Precision can be subdivided into three categories: repeatability, intermediate precision and reproducibility (Huber 1998, Peters *et al.* 2007).

#### REPEATABILITY

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

#### INTERMEDIATE PRECISION

Intermediate precision expresses within-laboratories variations: different days, different analysts or different equipment. In a strict sense intermediate precision is the total precision under varied conditions, whereas so-called interassay.

#### REPRODUCIBILITY

Reproducibility expresses the precision between laboratories (collaborative studies, usually applied to standardization of methodology) (Peters *et al.* 2007).

### 4.3.5. ACCURACY

Accuracy is the closeness of agreement between a test result and the accepted reference value of the property being measured. According to Thompson *et al.* (2002) accuracy is typically determined by comparing the response of the method to a reference material, comparing the response of a reference method or by spiking and recovery.

#### CERTIFIED REFERENCE MATERIALS (CRMS)

CRMs are traceable to international standards with a known uncertainty and therefore can be used to address all aspects of bias (method, laboratory, and within-laboratory) simultaneously, assuming that there is no matrix mismatch.

### Reference materials

Where CRMs are not available, can be used any material sufficiently well characterized for the purpose, bearing in mind always that while insignificant bias may not be proof of zero bias, significant bias on any material remains a cause for investigation.

### Use of a reference method

A reference method can be used to test for bias in another method under validation. This is a useful option when checking an alternative to, or modification of, an established standard method already validated and in use in the laboratory.

### Use of spiking/recovery

In the absence of reference materials, or to support reference material studies, bias can be investigated by spiking and recovery. A typical test material is analyzed by the method under validation both in its original state and after the addition (spiking) of a known mass of the analyte to the test portion. The difference between the two results as a proportion of the mass added is called the surrogate recovery or sometimes the marginal recovery.

# 5. MATERIALS AND METHODS

## 5.1. BEER SAMPLES

Eight fresh craft beers and two fresh commercial beers (Table 6) were used to examine the effect of natural aging. The craft beers are not pasteurized or filtered and made exclusively of natural raw materials. The commercial ale is pasteurized but not filtered and the commercial lager is pasteurized and filtered. Beers were stored for six months at 25 °C in the dark:

Table 6 - Beer Samples

| Craft Beer | | | | |
|---|---|---|---|---|
| **Nomenclature** | | **Recipe** | **Bottle** | **Caps.** |
| A | A33 | Weiss | 33 cL | metal |
| | A75 | | 75 cL | cork |
| B | B33 | Pilsner | 33 cL | metal |
| | B75 | | 75 cL | cork |
| C | C33 | Stout | 33 cL | metal |
| | C75 | | 75 cL | cork |
| D | D33 | Red Ale | 33 cL | metal |
| | D75 | | 75 cL | cork |
| **Commercial Beer** | | | | |
| Ale | | Weiss | 50 cL | metal |
| Lager | | Pilsner | 25 cL | metal |

## 5.3. CHEMICALS AND REAGENTS

Table 7 - Chemicals and reagents used

| Analyte | Supplier | *cat. Nº* | Purity, p/% |
|---|---|---|---|
| Ethanol | Fisher | 64-17-5 | ≥99.8 |
| Glycerol | Himedia | 56-81-5 | 99.5 |
| Glucose | Fisher | 50-99-7 | |
| Frutose | Panreac | 57-48-7 | 98 |
| Maltose | Fisher | 69-79-4 | |
| Sulphuric Acid | | 7664-93-9 | |
| Tartaric Acid | Sigma | 87-69-4 | 99.5 |
| Malic Acid | Acros Organics | 97-67-6 | 99 |
| Latic Acid | Fluka | 79-33-4 | 85-90 |
| Fumaric Acid | Fluka | 110-17-8 | ≥99 |
| Acetic Acid | Sigma | 64-19-7 | |
| Citric Acid | Panreac | 77-92-9 | 99.5 |
| 4-methylpentan-2-one | Fluka | 108-10-1 | ≥99.7 |
| Ethyl butyrate | Aldrich | 105-54-4 | 99 |
| Ethyl 2-methylbutyrate | Aldrich | 7452-79-1 | 99 |
| Ethyl 3-methylbutyrate | Aldrich | 108-64-5 | 98 |
| Hexanal | Fluka | 66-25-1 | |
| Isoamyl acetate | Aldrich | 123-92-2 | ≥99 |
| Ethyl hexanoate | Aldrich | 123-66-0 | ≥99 |
| Hexyl acetate | Aldrich | 142-92-7 | 99 |

Table 7 – (Continued) Chemicals and reagents used

| Analyte | Supplier | *cat. Nº* | Purity, p/% |
|---|---|---|---|
| 3-methyl-1-pentanol | Aldrich | 589-35-5 | 99 |
| Ethyl lactate | Aldrich | 97-64-3 | 98 |
| 1-hexanol | Fluka | 111-27-3 | > 99.9 |
| *E*-3-hexen-1-ol | Aldrich | 928-97-2 | 98 |
| *Z*-3-hexeno-1-ol | Fluka | 928-96-1 | ≥ 98 |
| Ethyl octanoate | Aldrich | 106-32-1 | ≥99 |
| Linalool | Aldrich | 78-70-6 | 97 |
| Ethyl decanoate | Aldrich | 110-38-3 | ≥99 |
| Diethyl succinate | Aldrich | 123-25-1 | 99 |
| 2-terpineol | Merck | 98-55-5 | ≥ 98 |
| Citronelol | Aldrich | 106-22-9 | 95 |
| Nerol | Aldrich | 106-25-2 | 97 |
| 2-phenylethyl acetate | Fluka | 103-45-7 | > 99 |
| Geraniol | Aldrich | 106-24-1 | 98 |
| Guaiacol | Aldrich | 90-05-1 | 98 |
| 4-ethylphenol | Aldrich | 123-07-9 | 99 |
| Dichloromethane | Merck | 75-09-2 | ≥99.8 |
| Anydrous sodium sulphate | Panreac | 7757-82-6 | 99 |

## 5.4. Quantification of major compounds:

### 5.4.1. HPLC

Ethanol, glycerol, carbohydrates (glucose, maltose, fructose) were quantified by HPLC using a Jasco chromatograph equipped with a refractive index (RI) detector (Jasco 830-RI), UV–visible detector (Jasco 870-UV–visible) and a 87H Chrompack column (7.8 mm × 300 mm) at 60 °C. Five mmol/L of sulphuric acid was used as the eluent, at a flow rate of 0.7 mL/min and a sample volume of 20 μL.

Organic acids (succinic, malic, citric, tartaric, lactic and acetic) were quantified by the same equipment but the separation was performed at 80°C and the eluent at a flow rate of 0.5 mL/min. Ethanol, glycerol and the carbohydrates were identified using RI detector. UV–visible detector was used for the identification of the organic acids at a wavelength of 210 nm.

## 5.5. Quantification of minor compounds

### 5.5.1. Extraction of volatile compounds

In a 10 ml culture tube (*Pyrex*, *ref. 1636/26MP*), 8 ml of the solution prepared, 2.46 μg of internal standard and a magnetic stir bar (22.2 mm × 4.8 mm) were added.

Extractions were performed by stirring the sample with 400 µl of dichloromethane during 15 min, using a magnetic stirrer, according to Oliveira *et al.* (2006) After cooling at 0 °C during 10 min, the magnetic stir bar was removed and the organic phase was separated by centrifugation (RPM = 4000, 7 min, 7 °C) being the extract recovered into a vial, using a Pasteur pipette. Then, water residues in the aromatic extract were removed with anhydrous sodium sulphate and transferred to a new vial.

### 5.5.2. GC- MS

Gas chromatography analysis of volatile compounds was performed using a GC–MS constituted by a Varian 3400 Chromatograph and an *ion-trap* mass spectrometer Varian Saturn II. A 1 µl injection was made in a capillary column, coated with CP-Wax 52 CB (50 m × 0.25 mm i.d., 0.2 µm film thickness, Chrompack). The temperature of the injector (SPI, septum-equipped programmable temperature) was programmed from 20 °C to 250 °C, at 180 °C $min^{-1}$. The oven temperature was held at 40 °C, for 5 min, then programmed to rise from 40 °C to 250 °C, at 3 °C $min^{-1}$, then held 20 min at 250 °C and finally programmed to go from 250 °C to 255 °C at 1 °C $min^{-1}$. The carrier gas was helium N60 (Air Liquide) at 103 kPa, which corresponds to a linear speed of 15.5 cm $s^{-1}$ at 150 °C. The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 *m/z* to 360 *m/z*, and an acquisition rate of 610 ms.

## 5.6. VALIDATION OF THE EXTRACTION METHOD

### 5.6.1. SAMPLE

A global standard solution (100x concentrate) containing 4-methylpentan-2-one, ethyl butyrate, ethyl 2-methylbutyrate, ethyl 3-methylbutyrate, hexanal, isoamyl acetate, ethyl hexanoate, hexyl acetate, 3-methyl-1-pentanol, ethyl lactate, 1-hexanol, *E*-3-hexen-1-ol, *Z*-3-hexeno-1-ol, ethyl octanoate, linalool, ethyl decanoate, diethyl succinate, 2-terpineol, citronelol, nerol, 2-phenylethyl acetate, geraniol, guaiacol, 4-ethylphenol was prepared in a hydro-alcoholic solution (7% ethanol in Milli-Q water) using different concentration ranges of analytes.

### 5.6.2. CALIBRATION CURVES

Standards containing known amounts of the volatile compounds, 7% (v/v) ethanol, were extracted and analyzed following the proposed procedure. The range of concentrations tested was from 0.25 up to 5 mg/l depending on the chemical nature of the analytes. For each compound, seven concentrations (1:1, 1:3, 1:5, 1:7, 1:10, 1:50, 1:100) were plotted against their

corresponding relative chromatographic response areas to the internal standard. Three replicates were performed for each concentration level for the calibration, and the average peak area ratios (peak area of a compound to the internal standard) against the known concentrations of standards used were applied to construct the calibration curves, for each volatile compound. From each curve, the regression coefficient ($R^2$), linearity (slope and interception) and quantification and detection limits were calculated.

### 5.6.3. Repeatability/ Intermediate precision

Repeatability was evaluated by replicated analysis of five samples in the same conditions of the proposed method. The intermediate precision (intermediate repeatability) was examined as the repeatability but the samples were analyzed by another analyst, in another laboratory and in another equipment.

### 5.6.4. Accuracy

In the absence of reference materials, or to support reference material studies, accuracy can be investigated by spiking and recovery. In this case, a commercial beer was analyzed by the method under validation, both in its original state and after the addition (spiking) of a known mass of the analyte to the test portion.

### 5.6.5. Other Parameters

Other parameters were studied to evaluate the susceptibility of a method to changes that might occur during routine (use a different matrix or more time of extraction).

The effect of the matrix was evaluated using two samples with the same compounds of the initial solution, in an intermediate concentration, but in two different matrices (three replicates). One with 95 g/L ethanol (12% vol./vol.), 5g/L of tartaric acid, 7.5 g/L of glycerol and 2g/L of malic acid, mimicking a synthetic wine matrix and another one with 10 g/L of citric acid and 50 g/L of acetic acid mimicking a synthetic vinegar matrix.

Modeling a change that might occur during routine, three replicates of an extraction were done by stirring the sample with dichloromethane during 30 min.

## 5.7. SENSORY ANALYSIS

Descriptive tests on beers in maturation were carried out using an untrained panel of 10 members (3 women and 7 men). Ten beers were presented in one session for month (during six months) to the panellists. Aspect, aroma, taste and mouthfeel were each evaluated by scoring specific descriptors from 0 to 8 (Table 8). A score of 0 meant the particular descriptor was not present, whereas a score of 8 meant the particular descriptor was extremely strong.

Table 8 - Scoring chart presented to the panellists in the sensorial test

| **Appearance** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Observations |
|---|---|---|---|---|---|---|---|---|---|---|
| Color | | | | | | | | | | |
| Clarity / Turbidity | | | | | | | | | | |
| Foam | | | | | | | | | | |
| **Aroma/Odor** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Descriptors |
| Fruity | | | | | | | | | | |
| Solvent | | | | | | | | | | |
| Papery/cardboard | | | | | | | | | | |
| Red fruit | | | | | | | | | | |
| Caramelized | | | | | | | | | | |
| Sulphury | | | | | | | | | | |
| Floral (hop) | | | | | | | | | | |
| **Taste** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Descriptors |
| Sweet | | | | | | | | | | |
| Sour | | | | | | | | | | |
| Hoppy | | | | | | | | | | |
| Toasted | | | | | | | | | | |
| **Sensation** | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Descriptors |
| Astringent | | | | | | | | | | |
| Warming | | | | | | | | | | |
| Body | | | | | | | | | | |
| Carbonation | | | | | | | | | | |

# 6. Results and discussion

## 6.1. Validation of the Extraction Method

### 6.1.1. Calibration Curves

As explained before the linearity of the method was determined from the calibration curves, created by plotting analyte concentrations against the relative chromatographic response of the analyte to the internal standard. Table 9 shows the results obtained as seven concentrations were used for building the calibration graphs for each compound.

Table 9 - Calibration curves and Method Linearity data

| Compound | Intercept | Slope: Sensibility | $R^2$ | Range (µg/L) |
|---|---|---|---|---|
| 4-methylpentan-2-one | 9.129 | 0.688 | 0.991 | 2.5 - 248 |
| ethyl butyrate | 7.529 | 0.607 | 0.998 | 5.8 - 576 |
| ethyl 2-methylbutyrate | 3.707 | 1.242 | 0.996 | 2.5 - 248 |
| ethyl 3-methylbutyrate | 4.662 | 1.058 | 0.998 | 3.1 - 312 |
| hexanal | 2.937 | 0.387 | 0.994 | 2.6 - 260 |
| isoamyl acetate | 16.465 | 0.473 | 0.997 | 21.3 - 2132 |
| ethyl hexanoate | 10.910 | 0.708 | 0.996 | 9.6 - 964 |
| hexyl acetate | 10.973 | 0.587 | 0.982 | 2.8 - 276 |
| 3-methyl-1-pentanol | 2.090 | 0.211 | 0.983 | 25.6 - 256 |
| ethyl lactate | 1.257 | 0.020 | 0.962 | 113.2 - 1132 |
| 1-hexanol | 1.167 | 0.273 | 0.991 | 14.7 - 1473 |
| *E*-3-hexen-1-ol | 1.162 | 0.200 | 0.987 | 6.3 - 632 |
| *Z*-3-hexeno-1-ol | 1.537 | 0.192 | 0.988 | 7.2 - 720 |
| ethyl octanoate | 17.523 | 0.579 | 0.994 | 14.5 - 1448 |
| linalool | 1.648 | 0.594 | 0.997 | 4.8 - 476 |
| ethyl decanoate | 14.363 | 0.491 | 0.989 | 9.8 - 976 |
| diethyl succinate | 4.271 | 0.766 | 0.997 | 6.1 - 612 |
| 2-terpineol | 2.337 | 0.773 | 0.997 | 2.6 - 260 |
| citronelol | -1.441 | 0.725 | 0.998 | 2.7 - 272 |
| nerol | -1.450 | 0.545 | 0.991 | 3.0 - 304 |
| 2-phenylethyl acetate | -14.749 | 0.884 | 0.992 | 10.3 - 1032 |
| geraniol | -2.235 | 0.868 | 0.997 | 3.1 - 308 |
| guaiacol | -2.110 | 0.511 | 0.987 | 5.8 - 292 |
| 4-ethylphenol | -11.447 | 0.742 | 0.973 | 4.9 - 488 |

Except for a few cases, the linearity was satisfactory, with correlation coefficient ($r^2$) varying between 0.962 and 0.998 for ethyl lactate and citronellol respectively.

The slope of the calibration lines is a measure of method sensitivity and depends on both extraction efficiency and detector response for each compound (Ortega *et al.* 2001). Therefore, the worst sensitivities obtained correspond to the compounds with smaller slope, which was ethyl

lactate, and consequently the better sensitivities correspond to the compounds with biggest slope, which were ethyl 3-methylbutyrate and the ethyl 2-methylbutyrate (Table 9).

### 6.2. Detection and Quantification Limits (LD) (LQ)

The limits of detection (LDs) and limits of quantification (LQs) were calculated from the calibration graphs constructed for each volatile compound as 3 and 10 times the ratio between the standard deviation of a response (s) and the slope of the analytical curve (b) (Ribani *et al.* 2007). Results are presented in Table 10.

Table 10 - Detection and quantification limits of each compound

| Compound | LD (µg/L) | LQ (µg/L) |
|---|---|---|
| 4-methylpentan-2-one | 0.01 | 0.04 |
| ethyl butyrate | 0.02 | 0.1 |
| ethyl 2-methylbutyrate | 0.01 | 0.03 |
| ethyl 3-methylbutyrate | 0.01 | 0.04 |
| hexanal | 0.1 | 0.2 |
| isoamyl acetate | 0.03 | 0.1 |
| ethyl hexanoate | 0.01 | 0.03 |
| hexyl acetate | 0.02 | 0.1 |
| 3-methyl-1-pentanol | 0.2 | 0.5 |
| ethyl lactate | 1.3 | 4.0 |
| 1-hexanol | 0.1 | 0.4 |
| *E*-3-hexen-1-ol | 0.1 | 0.2 |
| *Z*-3-hexeno-1-ol | 0.1 | 0.2 |
| ethyl octanoate | 0.2 | 0.5 |
| linalool | 0.04 | 0.1 |
| ethyl decanoate | 0.1 | 0.3 |
| diethyl succinate | 0.1 | 0.2 |
| 2-terpineol | 0.04 | 0.1 |
| citronelol | 0.02 | 0.05 |
| nerol | 0.02 | 0.1 |
| 2-phenylethyl acetate | 0.1 | 0.2 |
| geraniol | 0.02 | 0.1 |
| guaiacol | 0.01 | 0.04 |
| 4-ethylphenol | 0.1 | 0.2 |

In broad terms, the detection limit is the smallest amount or concentration of analyte in the test sample that can be reliably distinguished from zero (Thompson *et al.* 2002). The Table 10 clearly shows low LDs ranging from 0.01 µg/L (ethyl 2-methylbutyrate) to 1.3 µg/L (ethyl lactate). The quantification limit is the lowest sample concentration that can be quantified with suitable bias and precision (Hartmann *et al.* 1998). Low LQs ranging from 0.03 µg/L (ethyl 2-methylbutyrate) to 4.0

µg/L (ethyl lactate) were achieved. The wide range of LDs and LQs observed may be related to the difference in chemical and physical properties of each compound (Weldegergis and Crouch 2008).

### 6.2.1. REPEATABILITY/ INTERMEDIATE PRECISION

Repeatability was estimated as percent relative standard deviation (%RSD) (Eq. 3) of the relative peak areas for five replicates (same conditions of the proposed method) and varied between 6.8 and 27.1% (Table 11).

$$RSD\ (\%) = \frac{s}{\bar{x}} \times 100 \qquad \text{(Eq. 3)}$$

s - standard deviation

$\bar{x}$ - mean

Table 11 - Repeatability and intermediate precision for each compound

| Compound | Repeatability %RSD | Intermediate precision %RSD |
|---|---|---|
| 4-methylpentan-2-one | 12.5 | 6.5 |
| ethyl butyrate | 11.9 | 5.3 |
| ethyl 2-methylbutyrate | 13.1 | 9.0 |
| ethyl 3-methylbutyrate | 8.5 | 5.3 |
| hexanal | 8.5 | 6.1 |
| isoamyl acetate | 6.8 | 4.5 |
| ethyl hexanoate | 7.5 | 3.3 |
| hexyl acetate | 24.4 | 3.8 |
| 3-methyl-1-pentanol | 18.2 | 6.3 |
| ethyl lactate | 26.7 | 8.4 |
| 1-hexanol | 17.0 | 5.0 |
| *E*-3-hexen-1-ol | 22.7 | 7.0 |
| *Z*-3-hexeno-1-ol | 27.1 | 6.8 |
| ethyl octanoate | 10.4 | 4.0 |
| linalool | 14.6 | 4.0 |
| ethyl decanoate | 10.6 | 11.2 |
| diethyl succinate | 13.1 | 3.3 |
| 2-terpineol | 9.1 | 4.9 |
| citronelol | 13.4 | 4.4 |
| nerol | 19.1 | 5.9 |
| 2-phenylethyl acetate | 9.0 | 3.4 |
| geraniol | 8.8 | 2.6 |
| guaiacol | 18.5 | 4.6 |
| 4-etilguaicol | 13.2 | 3.7 |

The intermediate precision was examined as the repeatability but the samples were analyzed by another analyst, in another laboratory and in another equipment and calculated in terms of %RSD. The results ranged between 2.6 and 11.2%.

The range of acceptable %RSD for validation depends largely on the type of samples for which the method is intended to be used. While for compound analysis in pharmaceutical quality control precision of better than 1% RSD is easily achieved, for biological samples the precision is more like 15% at the concentration limits and 10% at other concentration levels. For environmental and food samples, the precision is very much dependent on the sample matrix, the concentration of the analyte and on the analysis technique. It can vary between 2% and more than 20%. (Huber 1998). Therefore, it is possible to conclude that the results are good since only three compounds (*E*-3-hexen-1-ol, *Z*-3-hexene-1-ol, hexyl acetate, ethyl lactate) were above the 20%, but that does not mean they were not considered valid since there is not a well-defined limit.

### 6.2.2. MATRIX EFFECT

To study the matrix effect, two synthetic matrices were made: one with 12% ethanol, 5g/L of tartaric acid, 7,5 g/L of glycerol and 2g/L of malic acid, mimicking a synthetic wine matrix and another one with 10 g/L of citric acid and 50 g/L of acetic acid mimicking a synthetic vinegar matrix. Two samples were prepared (one for the synthetic vinegar and another for the synthetic wine) with the same compounds of the initial solution, in an intermediate concentration. Results are presented in Table 12 and 13.

Table 12 - Effect of a synthetic wine matrix

| Compound | Determined Concentration (µg/L) | Expected Concentration (µg/L) | Recovery (%) |
|---|---|---|---|
| 4-methylpentan-2-one | 47.3 ± 9.4 | 49.6 | 95.4 ± 19.0 |
| ethyl butyrate | 103.4 ± 10.9 | 115.2 | 89.8 ± 9.5 |
| ethyl 2-methylbutyrate | 36.6 ± 6.5 | 49.6 | 73.7 ± 13.1 |
| ethyl 3-methylbutyrate | 53.3 ± 5.0 | 62.4 | 85.5 ± 8.0 |
| hexanal | 48.8 ± 8.3 | 52.0 | 93.8 ± 15.9 |
| isoamyl acetate | 343.9 ± 49.3 | 426.4 | 80.7 ± 11.6 |
| ethyl hexanoate | 193.5 ± 25.0 | 192.8 | 100.3 ± 12.9 |
| hexyl acetate | 46.5 ± 14.5 | 55.2 | 84.3 ± 26.3 |
| 3-methyl-1-pentanol | 68.9 ± 16.0 | 51.2 | 134.5 ± 31.2 |
| ethyl lactate | 380.5 ± 142.1 | 226.4 | 168.1 ± 62.7 |
| 1-hexanol | 339.3 ± 54.7 | 294.4 | 115.3 ± 18.6 |
| *E*-3-hexen-1-ol | 155.2 ± 28.5 | 126.4 | 122.8 ± 22.6 |
| *Z*-3-hexeno-1-ol | 168.6 ± 31.2 | 144.0 | 117.1 ± 21.7 |
| ethyl octanoate | 178.1 ± 44.8 | 289.6 | 61.5 ± 15.5 |
| Lilalool | 88.7 ± 10.2 | 95.2 | 93.2 ± 10.7 |
| ethyl decanoate | 78.6 ± 41.1 | 195.2 | 40.2 ± 21.0 |
| diethyl succinate | 142.3 ± 13.1 | 122.4 | 116.2 ± 10.7 |
| 2-terpineol | 55.4 ± 5.4 | 52.0 | 106.4 ± 10.4 |
| citronelol | 50.9 ± 4.5 | 54.4 | 93.6 ± 8.2 |
| nerol | 66.4 ± 11.4 | 60.8 | 109.2 ± 18.7 |
| 2-phenylethyl acetate | 204.0 ± 36.4 | 206.4 | 98.8 ± 17.6 |
| geraniol | 63.6 ± 7.0 | 61.6 | 103.2 ± 11.3 |
| guaiacol | 72.6 ± 14.7 | 58.4 | 124.3 ± 25.1 |
| 4-ethylphenol | 111.6 ± 31.8 | 97.6 | 114.4 ± 32.5 |

Table 13 - Effect of a synthetic vinegar matrix

| Compound | Determined Concentration (µg/L) | Expected Concentration (µg/L) | Recovery (%) |
|---|---|---|---|
| 4-methylpentan-2-one | 43.2 ± 9.4 | 49.6 | 87.0 ± 19.0 |
| ethyl butyrate | 103.4 ± 10.9 | 115.2 | 89.7 ± 9.5 |
| ethyl 2-methylbutyrate | 35.0 ± 6.5 | 49.6 | 70.5 ± 13.1 |
| ethyl 3-methylbutyrate | 57.7 ± 5.0 | 62.4 | 92.5 ± 8.0 |
| hexanal | 70.3 ± 8.3 | 52.0 | 135.2 ± 15.9 |
| isoamyl acetate | 394.0 ± 49.3 | 426.4 | 92.4 ± 11.6 |
| ethyl hexanoate | 189.6 ± 25.0 | 192.8 | 98.4 ± 13.0 |
| hexyl acetate | 39.5 ± 14.5 | 55.2 | 71.6 ± 26.4 |
| 3-methyl-1-pentanol | 66.0 ± 16.0 | 51.2 | 128.9 ± 31.2 |
| ethyl lactate | 219.7 ± 127.3 | 226.4 | 97.0 ± 56.2 |
| 1-hexanol | 277.1 ± 54.7 | 294.4 | 94.1 ± 18.6 |
| *E*-3-hexen-1-ol | 111.9 ± 28.5 | 126.4 | 88.6 ± 22.5 |
| *Z*-3-hexeno-1-ol | 160.5 ± 31.2 | 144.0 | 111.5 ± 21.7 |
| ethyl octanoate | NA | 289.6 | NA |
| Lilalool | 90.8 ± 10.2 | 95.2 | 95.4 ± 10.7 |
| ethyl decanoate | 20.3 ± 41.0 | 195.2 | 10.4 ± 21.0 |
| diethyl succinate | 137.7 ± 13.1 | 122.4 | 112.5 ± 10.7 |
| 2-terpineol | 49.6 ± 5.4 | 52.0 | 95.3 ± 10.4 |
| citronelol | 48.2 ± 4.5 | 54.4 | 88.6 ± 8.2 |
| nerol | 61.9 ± 11.4 | 60.8 | 101.8 ± 18.7 |
| 2-phenylethyl acetate | 199.8 ± 36.4 | 206.4 | 96.8 ± 17.6 |
| geraniol | 58.6 ± 7.0 | 61.6 | 95.1 ± 11.3 |
| guaiacol | 57.5 ± 14.7 | 58.4 | 98.5 ± 25.1 |
| 4-ethylphenol | 82.3 ± 31.8 | 97.6 | 84.3 ± 32.5 |

The expected recovery depends on the sample matrix, the sample processing procedure and on the analytic concentration (Table 14) (Huber 1998) .

Table 14 - Analyte recovery at different concentrations. Source: AOAC (1993)

| Active Ingred. (%) | Analyte ratio | Unit | Mean Recovery (%) |
|---|---|---|---|
| 100 | 1 | 100% | 98-102 |
| ≥10 | $10^{-1}$ | 10% | 98-102 |
| ≥1 | $10^{-2}$ | 1% | 97-103 |
| ≥0.1 | $10^{-3}$ | 0,10% | 95-105 |
| 0.01 | $10^{-4}$ | 100 ppm | 90-107 |
| 0.001 | $10^{-5}$ | 10 ppm | 80-110 |
| 0.0001 | $10^{-6}$ | 1 ppm | 80-110 |
| 0.00001 | $10^{-7}$ | 100 ppb | 80-110 |
| 0.000001 | $10^{-8}$ | 10 ppb | 60-115 |
| 0.0000001 | $10^{-9}$ | 1 ppb | 40-120 |

Since the expected concentrations were between 50 and 400 ppb, the compound with a recovery between 80% and 110% should be considered acceptable. Considering the errors associated to the quantifications using a calibration curve, all the compounds were within the range acceptable except the ethyl decanoate for both the matrices, the ethyl octanoate for wine matrix and the hexanal for vinegar matrix (Table 12,13). The ethyl octanoate was not quantified in the vinegar matrix since the peak corresponding to acetic acid covered the peak of ethyl octanoate.

### 6.2.3. TIME EFFECT

To verify if the time used for agitation was sufficient to recover all the compounds was made a test using 30 min of stirring (instead of 15 min) and it was found that all the compounds have been recovered within the expected except for ethyl octanoate, ethyl decanoate and nerol (Table15).

Table 15 - Time effect

| Compound | Determined Concentration (µg/L) | Expected Concentration (µg/L) | Recovery (%) |
|---|---|---|---|
| 4-methylpentan-2-one | 43.2 ± 9.4 | 49.6 | 87.2 ± 19.0 |
| ethyl butyrate | 111.6 ± 10.9 | 115.2 | 96.9 ± 9.5 |
| ethyl 2-methylbutyrate | 41.9 ± 6.5 | 49.6 | 84.5 ± 13.1 |
| ethyl 3-methylbutyrate | 57.8 ± 5.0 | 62.4 | 92.7 ± 8.0 |
| hexanal | 51.6 ± 8.3 | 52.0 | 99.3 ± 15.9 |
| isoamyl acetate | 427.6 ± 49.3 | 426.4 | 100.3 ± 11.6 |
| ethyl hexanoate | 190.6 ± 25.0 | 192.8 | 98.9 ± 13.0 |
| hexyl acetate | 44.1 ± 14.5 | 55.2 | 80.0 ± 26.4 |
| 3-methyl-1-pentanol | 41.7 ± 15.9 | 51.2 | 81.4 ± 31.0 |
| ethyl lactate | 172.9 ± 122.7 | 226.4 | 76.4 ± 54.2 |
| 1-hexanol | 266.90 ± 54.7 | 294.4 | 90.7 ± 18.6 |
| *E*-3-hexen-1-ol | 113.7 ± 28.5 | 126.4 | 90.0 ± 22.5 |
| *Z*-3-hexeno-1-ol | 120.2 ± 31.2 | 144.0 | 83.5 ± 21.7 |
| ethyl octanoate | 136.5 ± 44.8 | 289.6 | 47.2 ± 15.5 |
| Lilalool | 94.1 ± 10.2 | 95.2 | 98.9 ± 10.7 |
| ethyl decanoate | 51.1 ± 41.1 | 195.2 | 26.2 ± 21.0 |
| diethyl succinate | 137.8 ± 13.1 | 122.4 | 112.6 ± 10.7 |
| 2-terpineol | 58.1 ± 5.4 | 52.0 | 111.7 ± 10.4 |
| citronelol | 50.2 ± 4.5 | 54.4 | 92.3 ± 8.2 |
| nerol | 78.9 ± 11.4 | 60.8 | 129.8 ± 18.8 |
| 2-phenylethyl acetate | 200.7 ± 36.4 | 206.4 | 97.2 ± 17.6 |
| geraniol | 74.7 ± 7.0 | 61.6 | 121.3 ± 11.3 |
| guaiacol | 65.5 ± 14.7 | 58.4 | 112.1 ± 25.1 |
| 4-ethylphenol | 65.7 ± 31.8 | 97.6 | 67.3 ± 32.5 |

As the ethyl octanoate and the ethyl decanoate have been showing a decrease in the concentration in the synthetic matrices as well in this assay, it can be assumed that this compound may have evaporated from the stock solution. For Nerol, subtracting the error value to the percent recovery it gets 111.0% which is very close to the range of acceptable values (80-110%). However it can be seen in the Table 17 that this compound is present in a very low concentration, in order of 60 ppb, so if we consider de range of values acceptable for concentrations in order of 10 ppb which is 60-115 % the recovery of nerol is acceptable.

### 6.2.4. ACCURACY

The spiking test was similar to the assay made to study the matrix effect but a real matrix was use (commercial beer) which means that some of the compounds were present in the matrix. The commercial beer, was analyzed by the method under validation in its original state and after the addition (spiking) of the compounds. As happened for the synthetic matrices, almost all compounds showed a recovery within the valid range (Table 16).

Table 16 - Spiking test

| Compound | Spiking | Expected | Recovery (%) |
|---|---|---|---|
| 4-methylpentan-2-one | 38.3 ± 9.4 | 49.6 | 77.3 ± 19. |
| ethyl butyrate | 95.8 ± 10.9 | 115.2 | 83.2 ± 9.5 |
| ethyl 2-methylbutyrate | 47.8 ± 6.5 | 49.6 | 96.3 ± 13.1 |
| ethyl 3-methylbutyrate | 54.4 ± 5.0 | 62.4 | 87.2 ± 8.0 |
| hexanal | NA | 52.0 | NA |
| isoamyl acetate | 490.0± 49.3 | 426.4 | 115.0 ± 11.6 |
| ethyl hexanoate | 209.1 ± 25.0 | 192.8 | 108.4 ± 13.0 |
| hexyl acetate | 64.1 ± 14.6 | 55.2 | 116.1 ± 26.4 |
| 3-methyl-1-pentanol | 70.4 ± 16.0 | 51.2 | 137.5 ± 31.2 |
| ethyl lactate | NA | 226.4 | NA |
| 1-hexanol | 367.0 ± 54.7 | 294.4 | 124.6 ± 18.6 |
| *E*-3-hexen-1-ol | 157.8 ± 28.5 | 126.4 | 124.8 ± 22.6 |
| *Z*-3-hexeno-1-ol | 189.1 ± 31.3 | 144.0 | 131.3 ± 21.7 |
| ethyl octanoate | 126.2 ± 44.8 | 289.6 | 43.6 ± 15.5 |
| Lilalool | 112.3 ± 10.2 | 95.2 | 117.9 ± 10.7 |
| ethyl decanoate | 32.0 ± 41.0 | 195.2 | 16.4 ± 21.0 |
| diethyl succinate | 138.6 ± 13.1 | 122.4 | 113.2 ± 10.7 |
| 2-terpineol | 68.7 ± 5.4 | 52.0 | 132.0 ± 10.4 |
| citronelol | 52.7 ± 4.5 | 54.4 | 96.8 ± 8.2 |
| nerol | 7871 ± 11.4 | 60.8 | 128.5 ± 18.8 |
| 2-phenylethyl acetate | 200.3 ± 36.4 | 206.4 | 97.1 ± 17.6 |
| geraniol | NA | 61.6 | NA |
| guaiacol | NA | 58.4 | NA |
| 4-ethylphenol | 87.5 ± 31.8 | 97.6 | 89.7 ± 32.5 |

The ethyl octanoate and the ethyl decanoate showed again a concentration much lower than expected leading to believe, once again, by a possible evaporation from the stock solution. Other compounds as the guaicol, geraniol, ethyl lactate and hexanal were not able to be quantified once the corresponding peaks were covered by the peaks of other compounds present in beer.

## 6.3. Sensorial Analysis - T0

Results from Fig. 11 show that the aromatic profile of beers in 33 cL bottles was very similar to the aromatic profile of the beers in 75 cL bottles, which enables the establishment of comparisons over time. Since these results refer to the initial time, that is the closest time to the end of the production, and the beer inside the 33 cL bottles is the same as in the 75 cL bottles, can be affirmed that the similarity observed shows a good accuracy of the tasting panelists. Therefore the testing scheme can be considered appropriate.

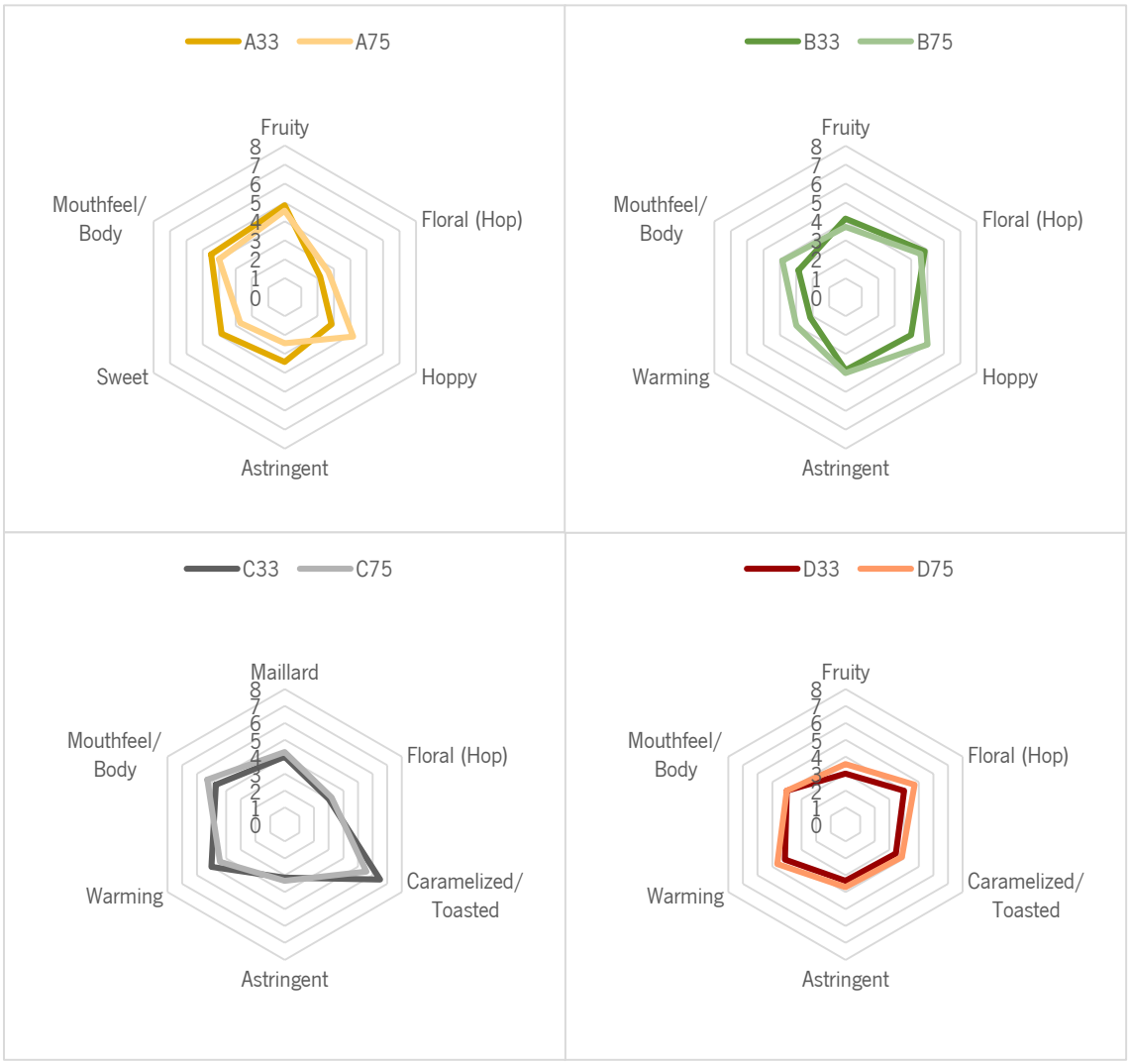


Fig. 11 - A comparison between the initial aromatic profiles of the studied beers in 33 cL capped bottles (A33, B33, C33 and D33) and 75 cL corked bottles (A75, B75, C75 and D75) for each recipe.

At time zero, the sample A was characterized by a fruity aroma and a lower astringency and floral aroma relative to the other beers (Fig. 12). This result is in line with the expected, since it is a weissbeer, being this recipe commonly characterized by fruity (banana aroma with esters are almost always present) and phenolic (aroma of nutmeg and cloves) descriptors and almost absence of floral aroma (Papazian 2006).

The sample B stands out by the floral aroma (Fig. 12). Since this Pilsner recipe is essentially characterized by hoppy aroma and taste as the original recipe created in Pilsen in Czech Republic (Papazian 2006).

The sample D had an identical profile to the sample B, differing by more caramelized and roasted aroma (Fig. 12). Red Ales have a bitterness and aroma from the hops of medium intensity as the sweetness and caramel. The fruity aromas are of low intensity but are still detected (Papazian 2006). Thus, the results of the sample D are within the expected given that it is a Red Ale.

The sample C stood out from all the others beer by the caramelized/toasted intense aroma and sweeter taste (Fig. 12). This Stout beer is characterized by an initial sweet taste (candy, chocolate and roasted coffee beans) with a crisp and distinct bitter in the end. The aromas of coffee and roasted flavors are usually predominant.

The fact of the panelists have been able to differentiate the beers going in line with the characteristics of each recipe shows once again a good sensorial accuracy and perception.

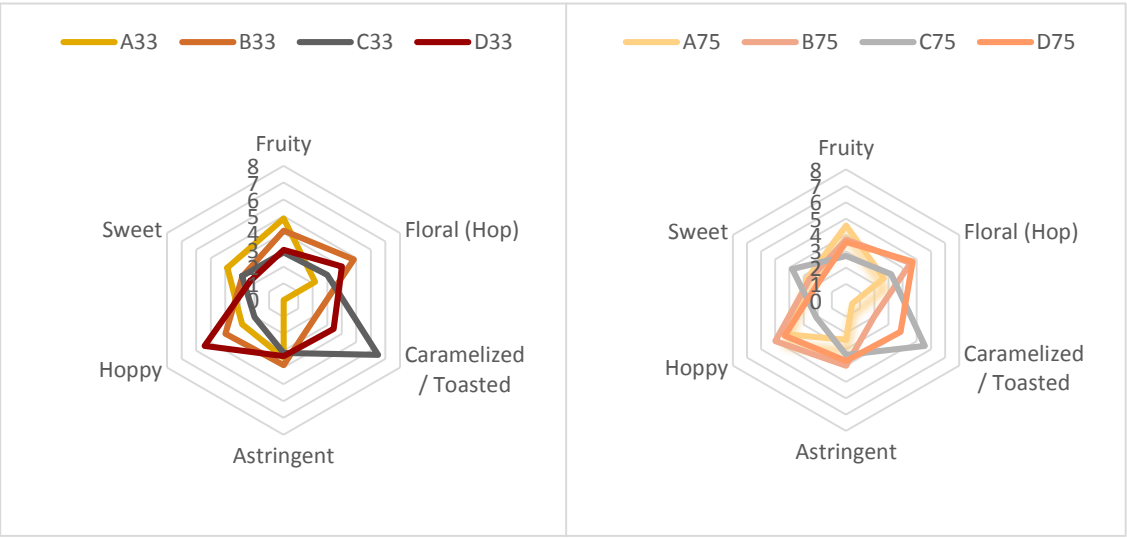


Fig. 12 - Comparison of the aromatic profile of each recipe in 33 cL capped bottles (A33, B33, C33 and D33) and 75 cL corked bottles (A75, B75, C75 and D75).

The commercial Ale showed a very similar profile to the sample A33 (Fig.13) which can be justified by the fact that both are Weiss beers and by the fact that this two beers have a common production feature – they both contain high amount of wheat and yeast in the bottle.

The similarity between the two profiles allows a comparison over ripening time, in other words, allows the detection of differences/similarities along the maturation time of the craft and commercial beer.

The sample B33 and the commercial Lager had a similar profile, but the sample B33 (Fig.13) had more intense aromas/flavors which can be justified by its craft beer nature. Only the warming parameter of sample B33 that was lower than commercial, due to lower alcohol content. Once again, the similarity allows us to take conclusions about the differences between a craft and a commercial beer during the maturation.

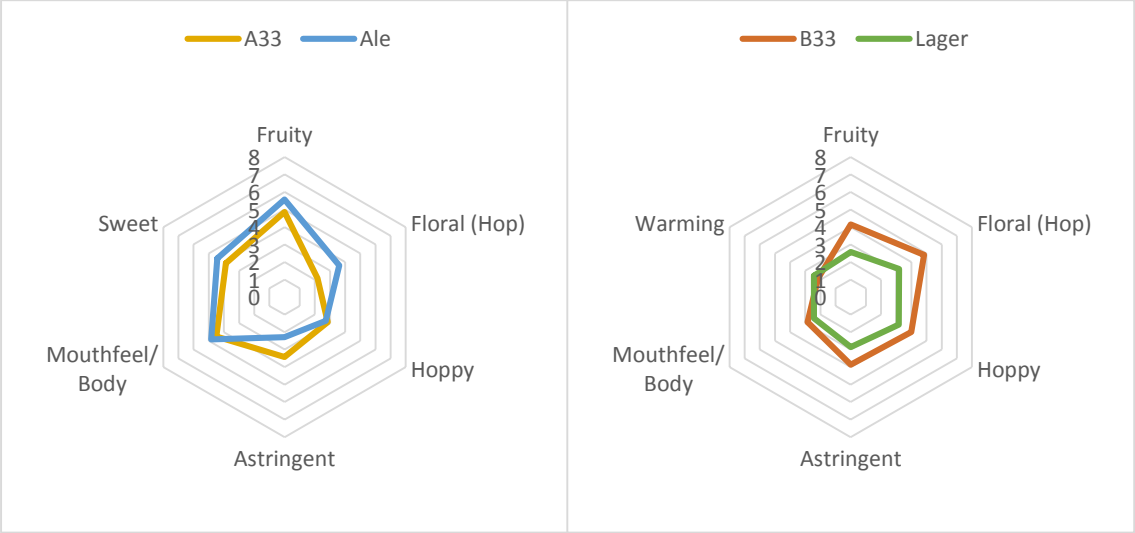


Fig. 13 -Comparison of the aromatic profile between two ales and two lagers craft and commercial beers.

## 6.4. EVALUATION OF THE DESCRIPTORS DURING SIX MONTHS

Fig. 14A shows a decrease of turbidity during the six months, for sample A33. This may be explained by coagulation and flocculation reactions occurring inside the bottle which clarify the beer. Flocculation tests for the strain used in the sample A allowed to conclude that this is a flocculent strain (Macieira 2013). The Fig. 15A shows that the exactly the opposite happens in the sample A75, for which there is an increase of turbidity. However the increase of turbidity does not necessarily mean that the reactions of coagulation and flocculation did not occur. It can though be related to the fact that when the bottle is opened there is a release of the gas mixing the entire content of the bottle and the yeast at the bottom to move through all the space, causing the turbidity of beer. It could be explained by the bigger size of the bottle and therefore has a bigger headspace, leading to greater internal pressure.

The color of the sample A33 remained constant during the six months however, the color of the sample A75 had a slight decrease in quality at the second and third months, going back to the initial value (Fig. 14A and 15A). The changes of food color during storage are explained by various mechanisms, which commonly include enzymatic or non-enzymatic oxidation of polyphenols and/or melanoidin substances formation (Šavel *et al.* 2010). Again, the fact of cork stopper being more permeable to gas exchanges and is more sensitive to oxygen contact could explain the reason why the sample A75 had a decrease in color quality. Foam quality roughly follows the same trend as the color quality, which supports the theory of occurrence of oxidation of polyphenols, since this reaction also affects the quality of the foam (Wunderlich and Back 2009).

It can be seen in the Fig. 14B, C and D the aroma, taste and mouthfeel descriptors had no significant changes, were considered constant over the six months. Observing the plots, it may seem that there is a variation in the intensity of descriptors however, taking into account the deviations, this variation is not considered significant and can be assumed that these descriptors remained constant.

The aroma and mouthfeel descriptors also remained constant for the sample A75 (Fig. 15C, D), but for taste some variations could be observed namely for sour (Fig. 15A). The main acids that contribute to sour flavor are acetic, citric, hydrochloric, malic and tartaric. Corroborating this result, the presence of acetic and malic acids could be detected above the threshold (Fig. 26) (Briggs *et al.* 2004).

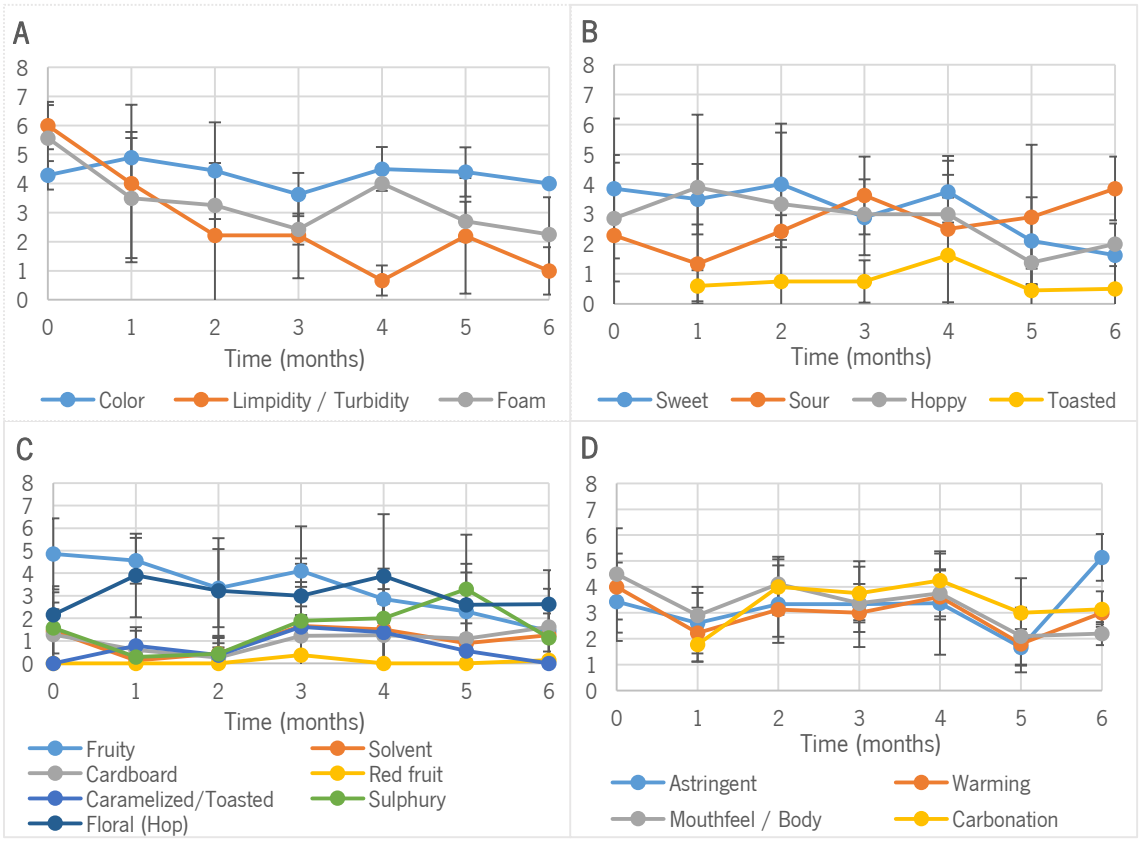


Fig. 14 - Evaluation of the different descriptors in the sample A33 (Weiss beer – 33 cL capped bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), and Mouthfeel (D).

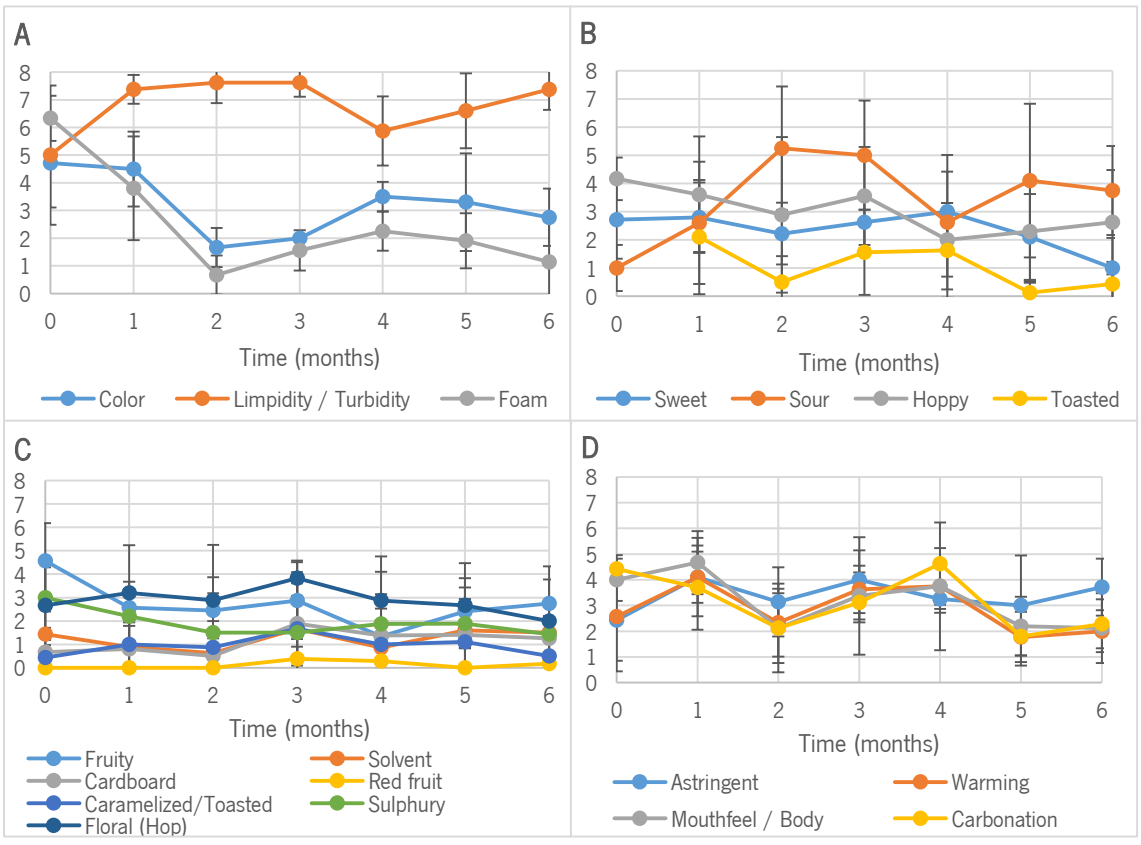


Fig. 15 - Evaluation of the different descriptors in the sample A75 (Weiss beer – 75 cL corked bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), and Mouthfeel (D).

As seen in the sample A33, was a decrease of turbidity in the sample B33 which can be justified for the reasons previously referred. The other descriptors of visual quality remained constant (Fig. 16A). As shown in Fig. 17A, all the visual descriptors remained constant for sample B75 (variations not significant taking into account the deviations).

Again, taking into account the deviations, as for sample B33, there were no significant changes, for the taste, aroma and mouthfeel descriptors (Fig. 16B, C, D and Fig. 17B, C, D). This is a positive result because it indicates that this recipe keeps important properties over the time regardless of cork or cap.

It is important to clarify the reason why the deviations are high. This can be related to the fact that the panel is not a trained panel. In addition, the panel of tasters was composed by three groups of people with different perceptions: a group that is used to evaluate beer, a group that is used to evaluate wine (being the wine aromas/flavors much more intense that the beer aromas/flavors) and a group of people who are not used to evaluating any type of beverage. Although the outliers have been eliminated, the deviations are still high due to different perceptions of each group. Nevertheless this panel was assembled to reflect the majority of the consumers, being considered representative of consumer perceived overall quality.

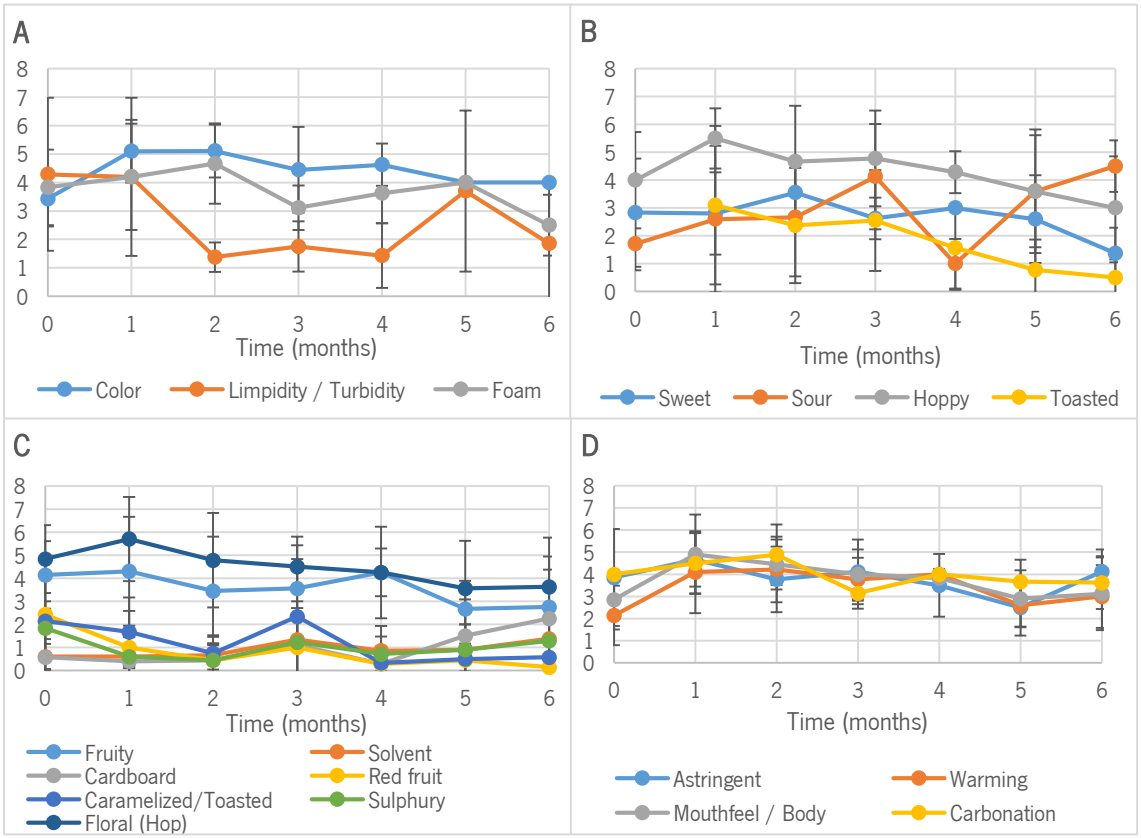


Fig. 16 - Evaluation of the different descriptors in the sample B33 (Pilsner beer – 33 cL capped bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

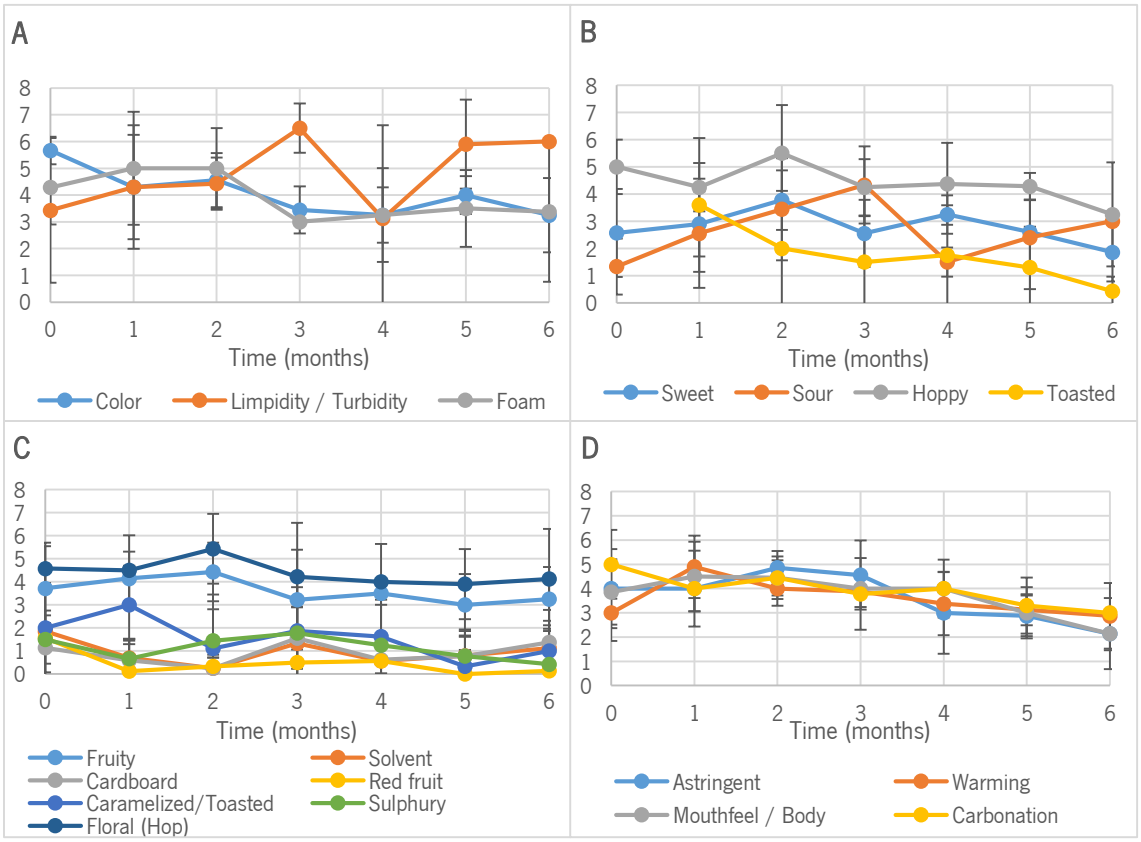


Fig. 17 - Evaluation of the different descriptors in the sample B75 (Pilsner beer – 75 cL corked bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

Results plotted in Fig. 18A and Fig. 19A show that the visual quality descriptors remained constant during the six months for sample C. Given that it is a stout beer, the sample C is a dark beers, making the visual aspects difficult to evaluate since the variations in color and turbidity are not perceptible.

In relation to the taste and mouthfeel descriptors (Fig. 18B, D and Fig. 19B, D) there were no significant variations taking into account deviations.

Analyzing both graphs (Fig. 18 and 19) it can be concluded that the caramelized /toasted aroma showed a slight decrease. According to Vanderhaegen *et al.* (2006) and the Fig. 7, the intensity of the caramel, burnt sugar and toffee-like aromas it's supposed to increase over time. Perhaps the caramel aroma perception was affected by changes in the conditions of the sensory evaluation environment or there may have been an increase of another taste/aroma that has hindered the perception of the caramelized flavor.

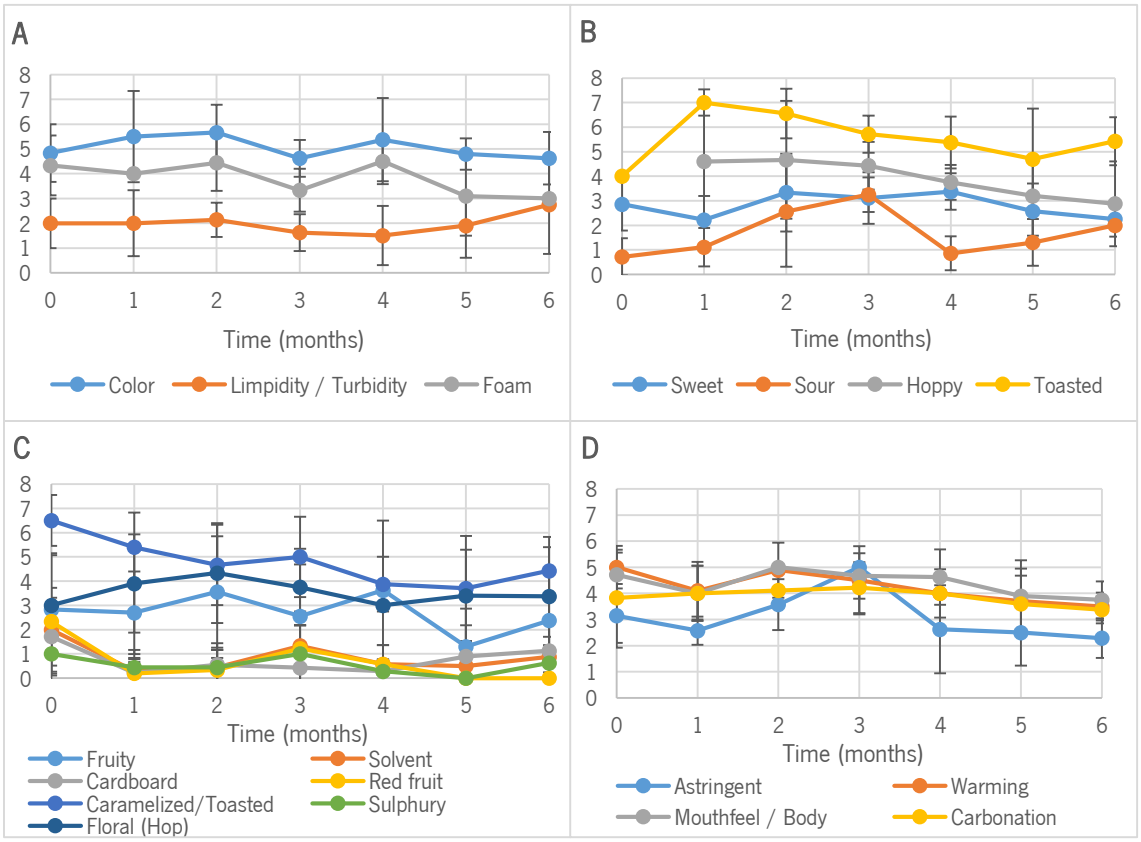


Fig. 18 - Evaluation of the different descriptors in the sample C33 (Stout beer – 33 cL capped bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

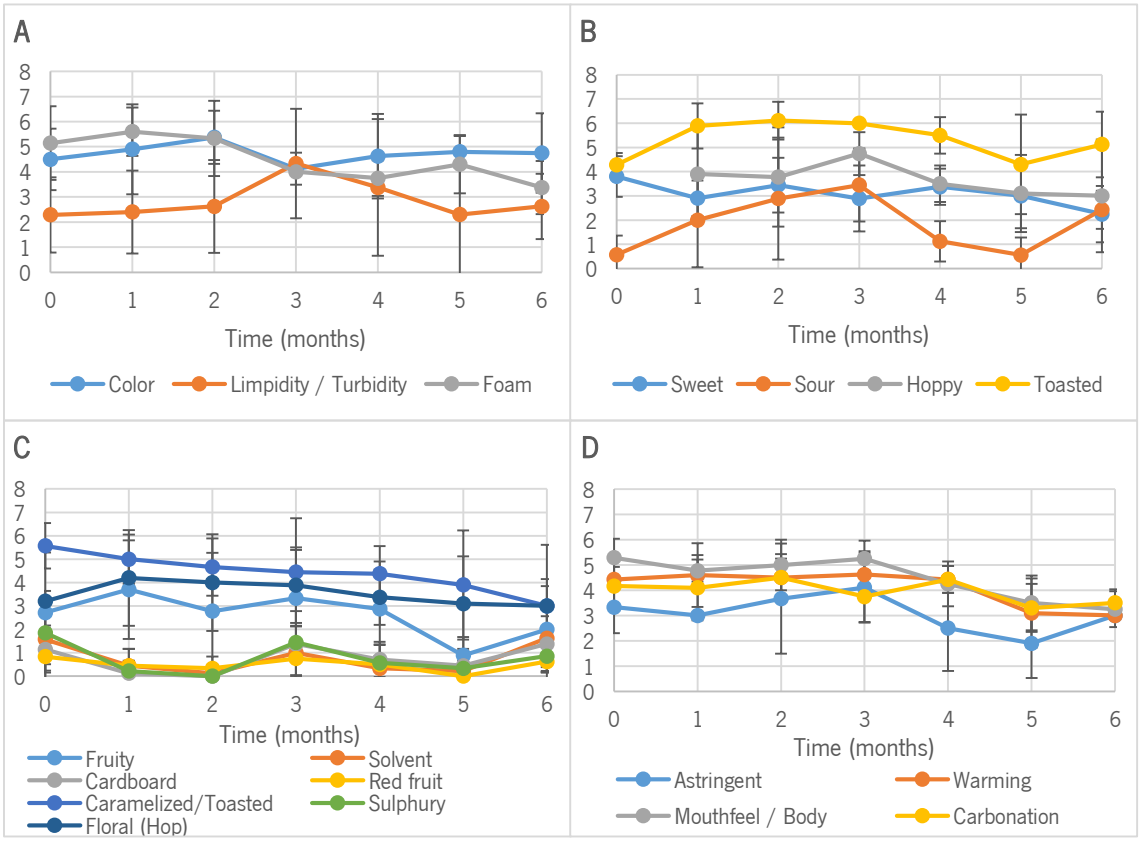


Fig. 19 - Evaluation of the different descriptors in the sample C75 (Stout beer – 75 cL corked bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

Despite the visual descriptors (essentially limpidity/turbidity) appear vary widely, the high deviations show that these variations are not significant. Therefore, it can be affirmed that all the descriptors remaind constant (taking into account the deviations) as for samples D33 and D75 (Fig. 20 and 21). Few conclusions can be made about the sensory analysis of this beer except that it is not sensitive to the variation of the storage conditions since it maintains its properties stable over time which is a great result.

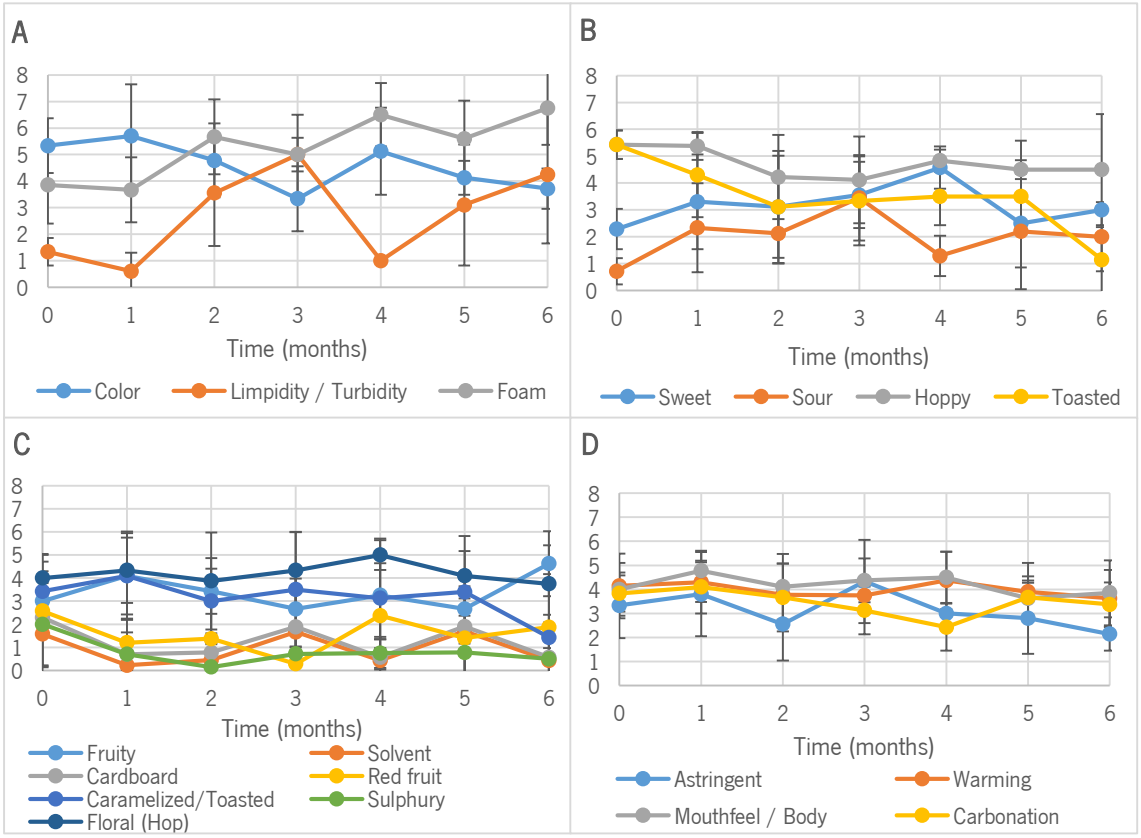


Fig. 20 - Evaluation of the different descriptors in the sample D33 for (Red Ale beer – 33 cL capped bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

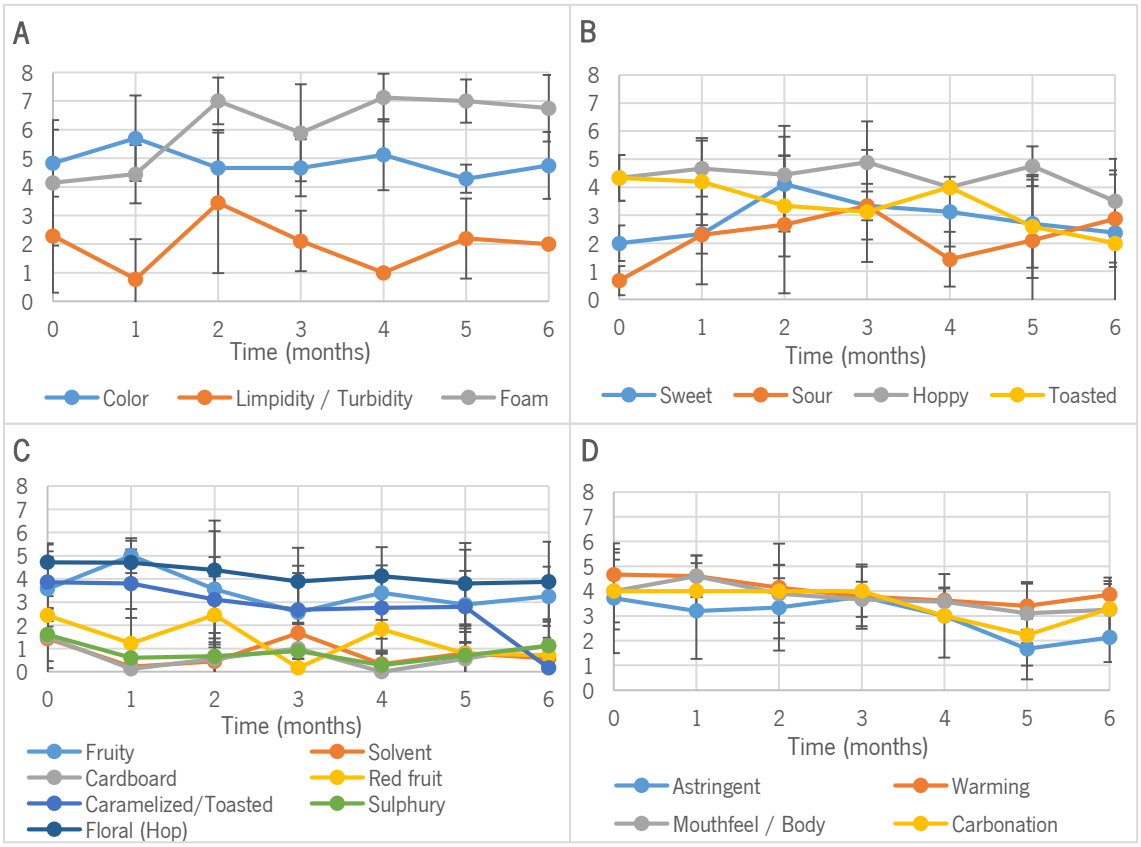


Fig. 21 - Evaluation of the different descriptors in the sample D75 (Red Ale beer – 75 cL corked bottle) for six months: Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

Fig. 22A presents results that indicate that the descriptors of the visual quality of the commercial ale had exactly the same behavior as the descriptors of the sample A33 (Fig. 14). As for sample A33, the other descriptors remained constant which may indicate that this is the typical behavior for a Weiss beer. These results indicates that commercial ale behaveed like a control with the aim to understand if the different variables of the craft beers (unfiltered and non-pasteurized) negatively influence beer quality. As the two had the same behavior can be conclude that the craft beer maintained the visual quality of a commercial beer, for the time tested, with the benefit of having most intense flavors and aromas.

Since the commercial lager is a type of beer which has suffered several preservation processes (pasteurization and filtration) and contains additives and preservatives, supposedly there is no change over time. This sample serves as a control to verify if the panel has the capability and sensitivity to always assign the same intensity over time for various descriptors. Analyzing the Fig. 23 it can be seen that the panel had this capability, despite of not being a trained panel and being constituted by people with different perceptions. This validates the panel and the results showed for the craft beers.

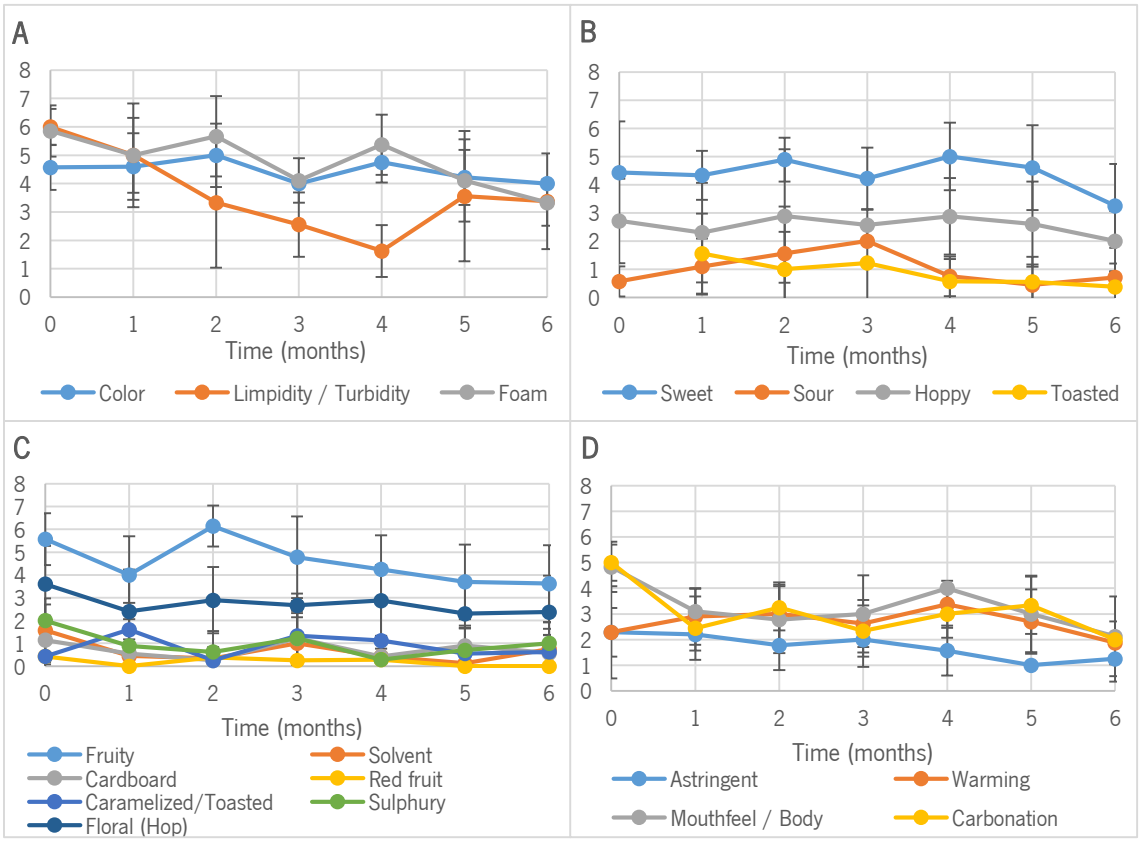


Fig. 22 - Evaluation of the different descriptors in commercial ale for six months : Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

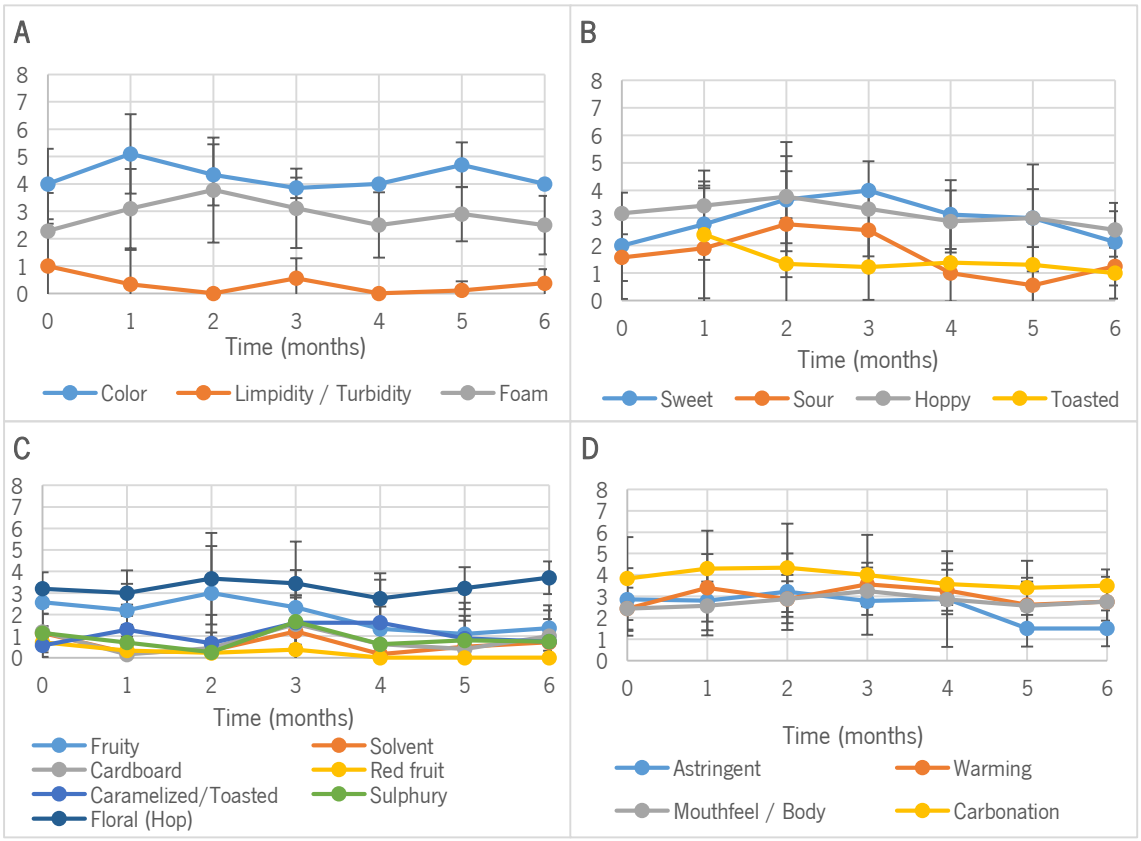


Fig. 23 - Evaluation of the different descriptors in commercial lager for six months : Visual Quality (A), Taste (B), Aroma (C), Mouthfeel (D).

## 6.5. QUANTIFICATION OF MAJOR COMPOUNDS

Figure 24 present the results for the ethanol quantification by HPLC during beer storage.

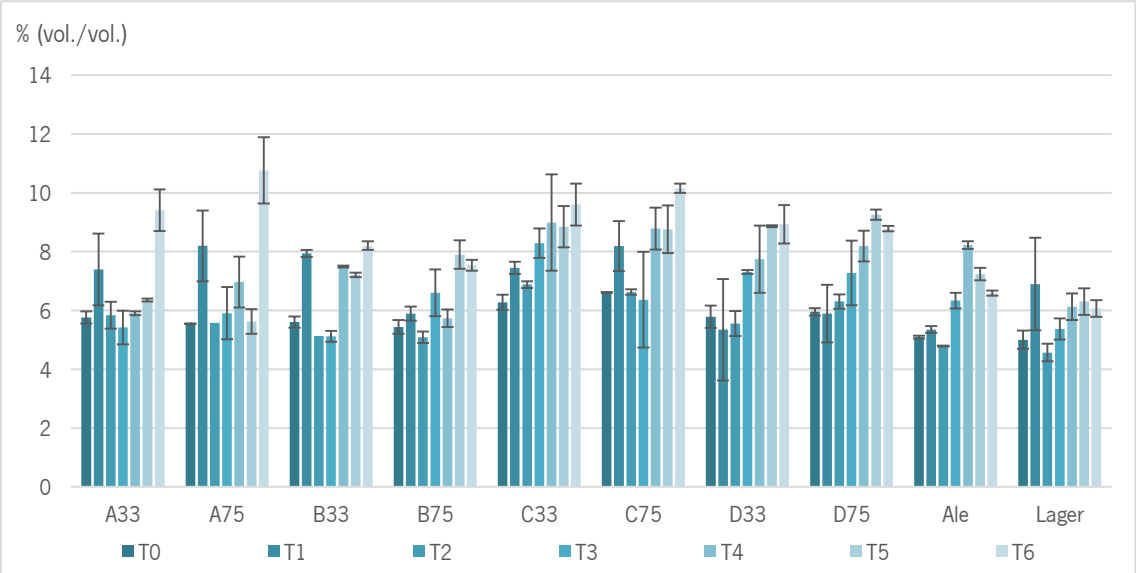


Fig. 24 – Ethanol concentration during beer storage.

These results are barely conclusive taking into account the deviations and the difficulty to affirm with certainty a tendency for ethanol concentration. To clear the doubts, some of the analysis should be repeated and should be continued over more time to determine the existence of a trend.

The values ranged between 5-11% (vol./vol.) for craft beers and between 5-8% (vol./vol.) for commercial ale.

The commercial lager works as a control since this is a pasteurized beer and therefore is not supposed to be observed changes in ethanol concentration. Taking into account the deviations observed for the commercial lager it can be assumed that the changes are not significant and concluded that the ethanol concentration remains constant (approximately 5.5%).

Figure 25 presents the results for glycerol concentration during beer storage.

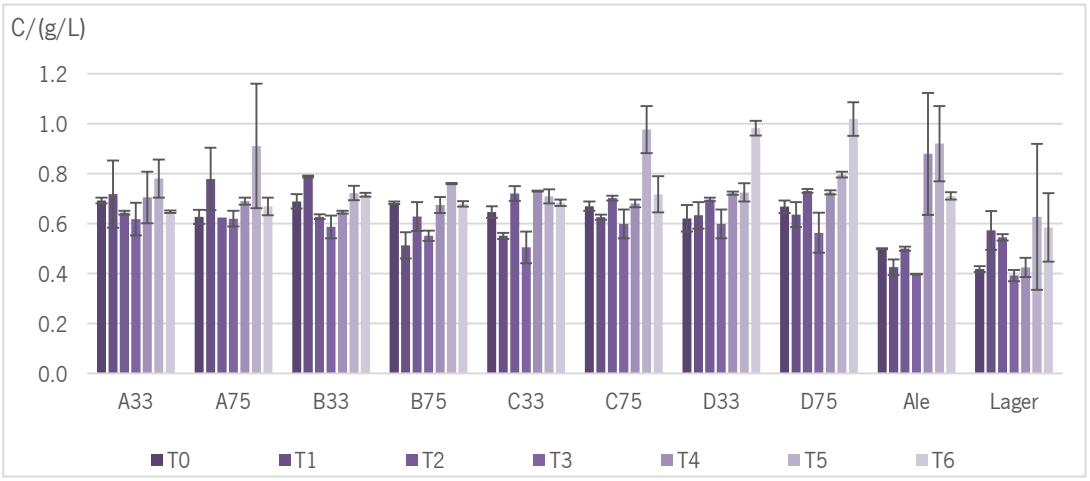


Fig. 25 - Glycerol concentration during beer storage.

Glycerol pyruvic fermentation occurs mainly in the early stages of the fermentation, when yeast use the substrates to multiply and synthesizes other essential metabolites from pyruvate (Nevoigt and Stahl 1997). This fact explains why, as expected, glycerol concentration remained constant (Fig. 24). For craft beers glycerol concentration was approximately 0.7 g/L while for the commercials was 0.55 g/L and 0.45 g/L (ale and lager respectively).

Figure 26 shows the results for the fermentable sugars concentration during beer storage.

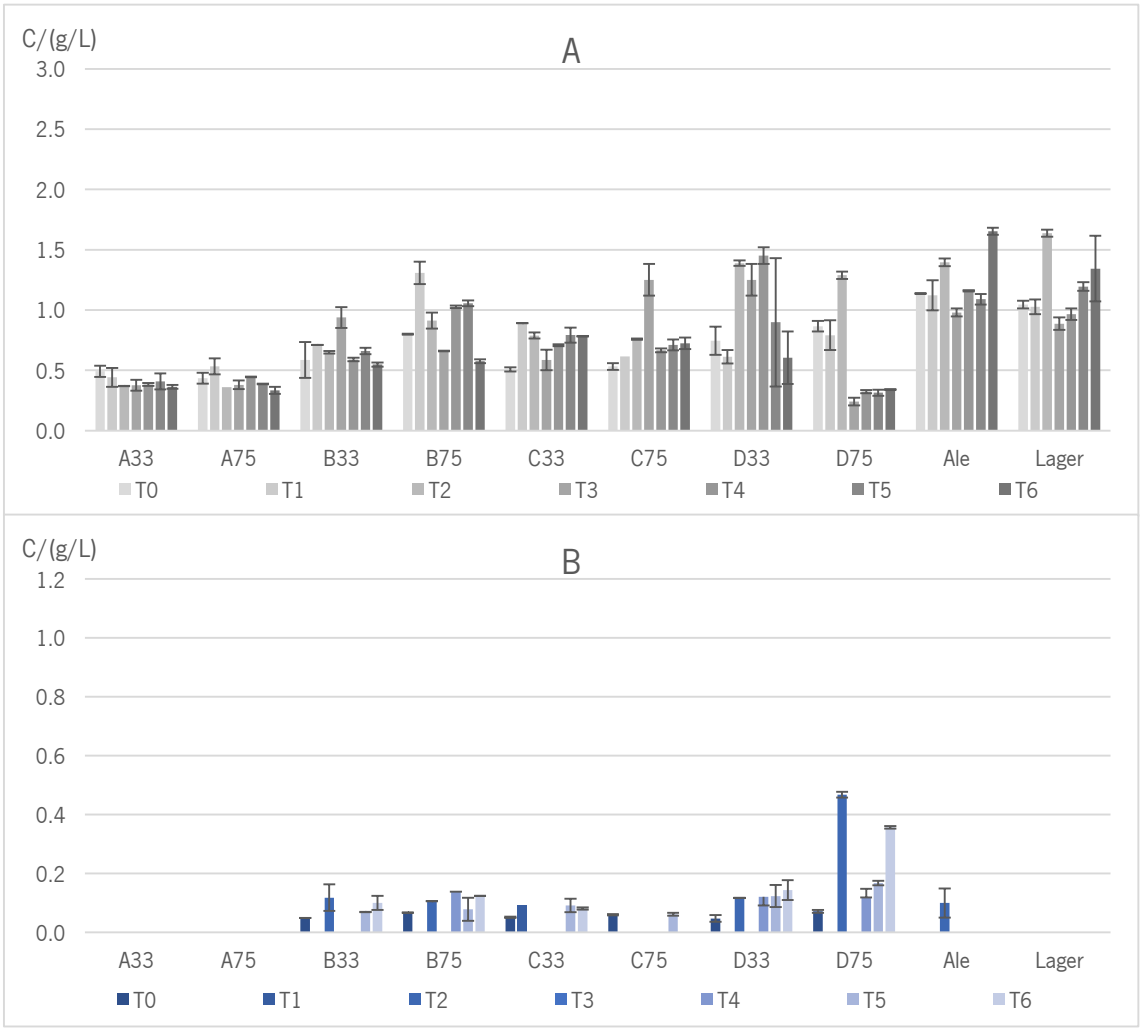


Fig. 26 - Sugars concentration during beer storage: maltose (A) and glucose (B).

It wouldn't be expected an increase of glucose concentration, as it can be seen for sample D75, since this is the sugar to be consumed by the yeast. There is the possibility of maltose is being hydrolyzed as glucose by maltases (α–Glucosidase). This presupposes a decrease of maltose concentration which can be seen again for sample D75.

There is not a clear trend in the maltose concentration, except for sample A where clearly the concentration remained constant, and there was not formation of glucose either (Fig. 26). The inconsistency of the values presented can be explained by some hypotheses. The quantification method used may not separate maltose from maltotriose so the concentrations shown could be the sum of the concentrations of the two compounds. It turns out that the appearance of glucose may be due to both the hydrolysis of maltose and maltotriose however this would imply, again, a decrease of concentration. The maltotriose and the maltose is hydrolyzed inside the cell so glucose concentration cannot be measured unless there is cell lysis. When the cellular lysis occurs the glucose that hadn't been fermented yet is liberated and the same happens for the maltose and maltotriose not hydrolyzed. Once released these could be transported again for other living cells, which could explain the inconstancy of the values.

Once craft brewing uses different yeast strains, it is natural that the yield of the fermentation is lower when compared to industrial strains. For this reason in the samples B, C and D (as shown in Fig. 26) there is a level of some fermentable sugars whiting the storage time. These results indicates that the used yeast is not capable of consuming all the sugars, where depending on the temperature this refermentation may occur in the bottle, overcarbonating beer in some cases.

Figure 27 presents the organic acids concentrations during beer storage, as well as the threshold level determined by Engan (1974) in Pilsner beers and typical concentrations determined in three studies (Coote and Kirsop 1974, Whiting 1976, Klopper *et al.* 1986) that include various types of commercial beers.

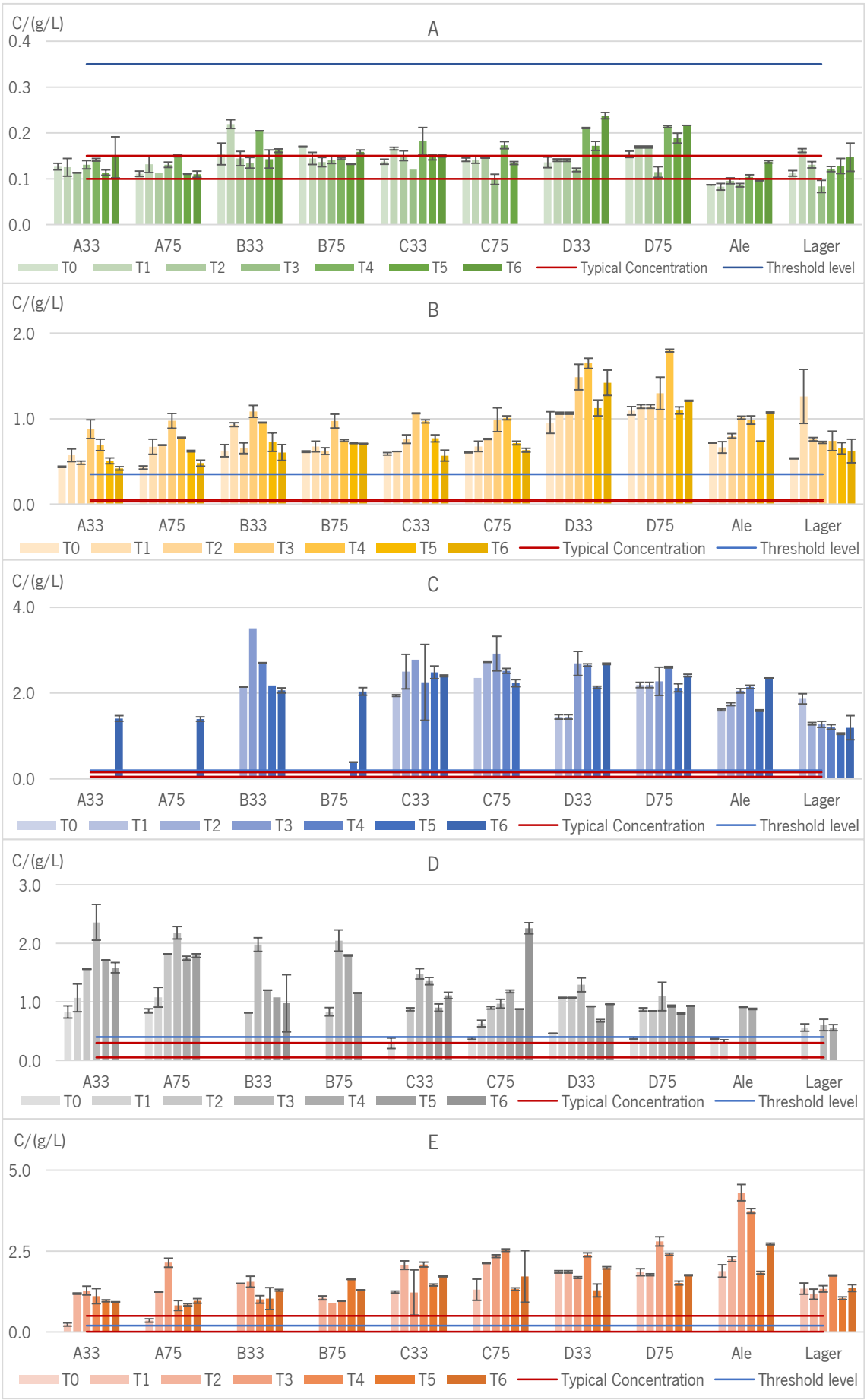


Fig. 27 - Organic acids concentrations during beer storage: citric (A), malic (B), succinic (C), lactic (D) and acetic acid (E); threshold level and typical concentrations reported in literature.

As it can be seen in Fig. 27(A) the citric acid concentration remained constant during beer storage, in concentrations raging form 0.08 to 0.22 g/L. Its content in beers is between 0.1–0.15 g/L and taking account de deviations, it can be assumed that the values are within the range expected.

Comparing the typical concentrations with the concentrations found in beer (Fig. 27) it can be seen that all the organic acids concentrations (except citric) are higher than expected. The concentrations are also above the threshold level, except citric acid. Being craft beers these beers have a higher amount overall yeast metabolites and as it can be seen in the graphs the commercial lager (pasteurized and filtered) has a lower amount of these acids. Also the contribution of the alpha acids of hops may interfere with the overall organic acid profiles of craft beers when compared to commercial beers.

## 6.6. Quantification of minor compounds

Gas chromatography analysis of minor compound was performed using a GC–MS. The compounds were extracted by the method proposed for the validation. Despite the validation has been successful, most of the compounds used for validation were not present in the analyzed beers. Therefore the concentrations presented in Tables 17, 18 and 19 are expressed as 4-nonanol (internal standard) equivalents. The relative concentrations of the compounds were calculated by relating the area of the internal standard to the area of the compound of interest.

The Tables 17, 18 and 19 do not show all the compound presented in the studied beers but that information can be found in the Annexes. The analytical determinations were carried out in duplicate. However the extraction method applied created a large emulsion in some of the beer and make it difficult to extract the dichloromethane. After some attempts the sample amount only left for one extraction that is why some of the beers do not have the deviations presented.

Analyzing the initial time we can compare the compounds found in each beer with the aromas characteristics of each recipe.

The Weiss beer (craft and commercial) had the higher esters concentration, essentially isoamyl acetate with a value of 1853.3 ± 57.9 µg/L and 1965.7 ± 28.7 µg/L for craft (samples A33 and A75 respectively) and 2223.7 µg/L for commercial (Table 17). Being isoamyl acetate a very important compound in wheat beers, is visible that commercial example had a higher amount, and the concentration increased during storage time, while for craft beer this value decreased. The same was verified for phenolic compounds like 4-vinylguaicol with a value of 1492.2 ± 16.1 µg/L and 1835.6 ± 86.6 µg/L for craft (samples A33 and A75 respectively) and 1666.5 µg/L for commercial. These results are in line with the sensory analysis and the literature, since this recipe is commonly characterized by fruity (banana aroma with esters are almost always present) and phenolic (aroma of nutmeg and cloves) descriptors (Papazian 2006).

The presence of the monoterpene linalool in high concentration (57.5 ± 0.9 µg/L and 53.4 ± 1.2 µg/L for samples B33 and B75 respectively) for Pilsner and above the threshold (25.2 µg/L) are also in accordance with the sensory analysis and literature (Table 18). Pilsner beer is essentially characterized by hoppy aroma and taste (Papazian 2006) and it was found by Fritsch and Schieberle (2005) that linalool is a determining odorant in Pilsner-type beers.

The commercial lager had much lower concentration of all compounds compared to other beers (Table 18), which also goes in line with the sensory analysis, where it was found that this is a beer with much less flavors and aromas.

Stout beer also stand out by the presence of a group of compounds, pyrazines (Table 19). These heterocyclic molecules are responsible for sweet, candy floss, caramel and cereal roasted aromas (Vanderhaegen *et al.* 2006). This beer stood out from all the others beers in sensory analysis by the caramelized/roasted intense aroma and sweeter, characteristic aromas of a Stout beer (Papazian 2006).

It was conclued in the sensory analysis that the Red Ale had an identical profile to the Pilsner beer. This fact can also be seen for minor compounds (Table 19). Red Ales have a bitterness and aroma from the hops of medium intensity. In fact linalool is present in a higher concentration than in the Weiss and Stout but smaller than in the Pilsner. The sweetness and caramel flavor are also of medium intensity (the fact of linalool being present in smaller amounts can allow identifying the presence of sweet taste).

Table 17 - Concentration of minor volatile compounds detected in the sample A (33 cL and 75 cL bottles) and in the commercial Ale by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | A33 | | | A75 | | | Commercial Ale | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | | |
| isoamyl acetate | 1853.3 ± 57.9 | 1247.2 ± 42.7 | ↓ | 1965.7 ± 28.7 | 765.5 ± 35.3 | ↓ | 2223.7 | 3180.2 ± 88.9 | ↑ | 1200 (A)c | Banana (C) |
| isobutyl acetate | 161.9 ± 14.5 | 181.1 ± 4.3 | = | 166.2 ± 4.5 | 131.5 ± 13.1 | ↓ | 177.2 | 237.5 ± 0.5 | ↑ | 1600(A)c | Banana, fruity (D) |
| ethyl butyrate | 49.1 ± 1.3 | 69.7 ± 1.0 | ↑ | | 54.6 ± 4.4 | ↑ | 64.3 | 108.4 ± 0.6 | ↑ | 20 (C)b | Papaya, sweetish, apple (B, D) |
| ethyl hexanoate | 97.3 ± 7.9 | 74.4 ± 5.4 | ↓ | 84.5 ± 1.5 | 84.1 ± 7.6 | = | 38.7 | 92.6 ± 1.7 | ↑ | 210 (A)c | Fruity, green apple (D, F) |
| ethyl lactate | 365.1 ± 130.2 | 844.5 ± 133.7 | ↑ | 436.0 ± 11.4 | 721.2 ± 56.0 | ↑ | 23.4 | 30.7 ± 0.6 | ↑ | 25000 (A)c | Strawberry, raspberry, perfumed (D, F) |
| ethyl octanoate | 120.9 ± 23.3 | 38.4 ± 1.4 | ↓ | 113.8 ± 4.1 | 86.7 ± 5.4 | ↓ | | 15.4 ± 0.2 | ↑ | 900(A)c | Apple, fruity, sweet (D, F) |
| diethyl succinate | 4.6 ± 0.8 | 29.8 ± 6.9 | ↑ | 4.1 ± 0.2 | 39.0 ± 0.5 | ↑ | | | | 1200(A)c | |
| 2-phenylethyl acetate | 458.4 ± 1.3 | 439.4 ± 18.0 | = | 456.6 ± 15.5 | 318.0 ± 18.7 | ↓ | 789.9 | 881.1 ± 8.4 | ↑ | 250 (C)b | Apple, honey, roses, flowery (D) (F) |
| 2-methyl-1-propanol | 629.6 ± 10.2 | 1368.6 ± 265.3 | ↑ | 762.6 ± 3.8 | 1001.7 ± 215.2 | ↑ | 551.7 | 563.7 ± 31.1 | = | - | |
| 1-hexanol | 20.9 ± 1.0 | 31.4 ± 3.5 | ↑ | 21.7 ± 1.5 | 27.5 ± 2.6 | ↑ | | 8.3 ± 0.7 | ↑ | 8000 (C)b | |
| 3-etoxi-1-propanol | 6.4 ± 0.1 | 14.1 ± 2.6 | ↑ | 6.9 ± 0.4 | 11.2 ± 2.8 | ↑ | | | | - | |
| 2-methyl-1-butanol + 3-methyl-1-butanol | 907.5 ± 5.0 | 17561.9 ± 2856.7 | ↑ | 1032.5 ± 0.5 | 12320.9 ± 2076.4 | ↑ | 8422.9 | 9709.5 ± 652.2 | ↑ | - | |
| furfuryl alcohol | 37.7 ± 1.9 | 94.0 ± 17.1 | ↑ | 40.1 ± 2.3 | 74.2 ± 7.9 | ↑ | 40.7 | 54.0 ± 1.2 | ↑ | 1000 (D)a | Moldy hay (E) |
| 2-phenylethanol | 5806.9 ± 174.6 | 9986.8 ± 1851.5 | ↑ | 5744.4 ± 179.5 | 7599.8 ± 875.3 | ↑ | 5769.5 | 5668.6 ± 70.7 | ↓ | | |
| methionol | 151.6 ± 4.5 | 320.2 ± 55.0 | ↑ | 169.0 ± 0.3 | 259.5 ± 27.5 | ↑ | 172.7 | 193.1 ± 3.4 | ↑ | 36 (B)a | Cooked potato-like (B) |
| tyrosol | 8.7 ± 1.6 | | ↓ | 12.5 ± 1.9 | | ↓ | 18.4 | | ↓ | - | Bitter, chemical (F) |
| tryptophol | 554.6 ± 1.3 | 430.4 ± 10.2 | ↓ | 469.0 ± 56.2 | 478.5 ± 52.3 | = | 761.9 | 528.3 ± 20.0 | ↓ | | |
| linalool | 4.1 ± 1.0 | | ↓ | 4.2 ± 0.5 | | ↓ | 2.1 | | ↓ | 25.2 (H)d | Hoppy (I) |
| g-nonalactone | 40.2 ± 0.3 | 170.6 ± 0.4 | ↑ | 48.0 ± 4.7 | 164.9 ± 20.3 | ↑ | 32.4 | 46.3 ±0.4 | ↑ | | |
| 4-ethylguaiacol | 8.0 ± 1.0 | | ↓ | 10.2 ± 2.0 | | ↓ | 38.0 | 42.2 ± 0.5 | ↑ | | |
| 4-vinylphenol | 34.7 ± 24.3 | | ↓ | 8.3 ± 1.5 | | ↓ | 182.5 | 167.6 ± 2.5 | ↓ | | |
| 4-ethylphenol | 328.2 ± 70.3 | 548.5 ± 41.3 | ↑ | 420.1 ± 14.0 | 496.9 ± 3.5 | ↑ | | | | | |
| 4-vinylguaiacol | 1492.2 ± 16.1 | 1100.7 ± 120.4 | ↓ | 1835.6± 86.6 | 979.6 ± 39.6 | ↓ | 1666.5 | 1209.7 ± 16.6 | ↓ | 21 (B)a | Clove-like, smoky (B) |
| 3-methyl-butyric acid | 37.3 ± 0.5 | 102.6 ± 10.1 | ↑ | 48.4 ± 5.6 | 86.8 ± 4.6 | ↑ | 35.2 | 44.4 ± 0.5 | ↑ | 33 (F)d | Sweaty cheese (D, B) |
| hexanoic acid | 214.3 ± 6.5 | 584.2 ± 91.6 | ↑ | 238.5 ± 18.7 | 471.1 ± 57.2 | ↑ | 273.7 | 389.6 ± 14.4 | ↑ | 420 (F)d | Vegetable oil, cheese, sweaty (D, F) |
| decanoic acid | 25.4 ± 7.5 | | ↓ | 64.7 ± 16.4 | | ↓ | 802.0 | 1380.0 ± 65.9 | ↑ | 1000 (F)d | Wax, tallow, rancid, soap, fatty (D, F) |
| octanoic acid | 1249.1 ± 76.7 | 2176.2 ± 88.6 | ↑ | 1483.7 ± 56.9 | 2116.8 ± 25.8 | ↑ | 2047.8 | 2765.8 ± 5.7 | ↑ | 500 (H)d | Vegetable oil rancid, harsh (D, F) |
| 2-methyl-propionic acid | 15.1 ± 2.0 | 62.4 ± 1.4 | ↑ | 17.7 ± 6.8 | 64.5 ± 1.3 | ↑ | 12.1 | 14.6 ± 1.2 | ↑ | 200000 (C)b | Sweat, bittercheese, rancid (D, F) |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase; ↓ decrease; = constant

Table 18 - Concentration of minor volatile compounds detected in the sample B (33 cL and 75 cL bottles) and in the commercial Lager by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | B33 | | | B75 | | | Comercial Lager | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0M | 6 M | | | |
| isoamyl acetate | 767.3 ± 40.3 | 453.0 ± 7.5 | ↓ | 710.7 ± 3.8 | 455.1 ± 21.4 | ↓ | 619.6 ± 89.3 | 784.2 ± 56.2 | ↑ | 1200 (A)c | Banana (C) |
| ethyl butyrate | 27.5 ± 2.2 | 78.0 ± 9.9 | ↑ | | 65.4 ± 6.0 | ↑ | 36.2 ± 4.9 | 56.0 ± 5.4 | ↑ | 20 (C)b | Papaya, sweetish, apple (B, D) |
| ethyl hexanoate | 144.9 ± 22.8 | 109.3 ± 15.5 | ↓ | 104.0 ± 0.5 | 85.5 ± 3.8 | ↓ | 30.4 ± 2.4 | 42.5 ± 3.6 | ↑ | 210 (A)c | Fruity, green apple (D, F) |
| ethyl lactate | 17.0 ± 4.6 | 81.0 ± 23.5 | ↑ | 8.6 ± 0.5 | 28.6 ± 0.1 | ↑ | | | | 25000 (A)c | Strawberry, raspberry, perfumed (D, F) |
| ethyl octanoate | 242.3 ± 26.3 | 129.1 ± 39.2 | ↓ | 230.5 ± 1.5 | 81.7 ± 3.8 | ↓ | | | | 900(A)c | Apple, fruity, sweet (D, F) |
| ethyl decanoate | 76.6 ± 42.1 | 18.5 ± 2.9 | ↓ | 46.4 ± 0.3 | 12.6 ± 1.5 | ↓ | | | | 570(A)c | |
| diethyl succinate | 16.4 ± 5.9 | 47.3 ± 8.2 | ↑ | 12.7 ± 0.9 | 31.4 ± 3.6 | ↑ | | | | 1200(A)c | |
| 2-phenylethyl acetate | 377.4 ± 14.4 | 251.2 ± 2.1 | ↓ | 390.5 ± 9.8 | 256.4 ± 20.6 | ↓ | 257.2 ± 43.2 | 380.7 ± 30.6 | ↑ | 250 (C)b | Apple, honey, roses, flowery (D, F) |
| 2-methyl-1-propanol | 226.0 ± 53.8 | 482.2 ± 85.3 | ↑ | 173.9 ± 0.4 | 287.5 ± 20.2 | ↑ | 134.8 ± 22.3 | 326.8 ± 98.1 | ↑ | - | |
| 1-hexanol | 16.3 ± 2.5 | 26.7 ± 2.8 | ↑ | 11.9 ± 0.0 | 16.4 ± 1.4 | ↑ | | | | 8000 (C)b | |
| 2-methyl-1-butanol + 3-methyl-1-butanol | 7036.7 ± 1877.7 | 14049.3 ± 2161.5 | ↑ | 5653.2 ± 89.1 | 8570.4 ± 715.8 | ↑ | 4149.7 ± 684.7 | 8936.9 ± 2408.4 | ↑ | - | |
| 1-octanol | 31.9 ± 4.6 | 28.0 ± 0.4 | = | 25.9 ± 1.0 | 26.5 ± 1.5 | = | | 6.8 ± 1.0 | ↑ | 900 (F)c | Coconut, walnut, oily (F) |
| furfuryl alcohol | 57.6 ± 11.9 | 120.7 ± 9.3 | ↑ | 40.4 ± 0.0 | 73.4 ± 6.7 | ↑ | 27.0 ± 4.7 | 33.9 ± 7.4 | ↑ | 1000 (D)a | Moldy hay (E) |
| 2-phenylethanol | 7016.8 ± 765.1 | 13262.0 ± 844.6 | ↑ | 6201.1 ± 134.4 | 8492.1 ± 867.3 | ↑ | 4405.8 ± 584.2 | 7175.8 ± 1589.1 | ↑ | | |
| 1-butanol | 16.9 ± 5.3 | | ↓ | 13.4 ± 0.5 | | ↓ | 13.6 ± 2.4 | | ↓ | 590 (B)a | Malty, solvent-like, fusel (D, B) |
| methionol | 24.5 ± 7.0 | 76.8 ± 6.4 | ↑ | 20.0 ± 0.2 | 48.4 ± 2.1 | ↑ | 24.8 ± 3.8 | 57.6 ± 18.9 | ↑ | 36 (B)a | Cooked potato-like (B) |
| tyrosol | 48.4 ± 17.9 | | ↓ | 43.9 ± 2.8 | | ↓ | 16.2 ± 9.7 | | ↓ | - | Bitter, chemical (F) |
| tryptophol | 57.3 ± 24.2 | | ↓ | 54.0 ± 4.6 | | ↓ | 41.5 ± 5.4 | | ↓ | | |
| linalool | 57.5 ± 0.9 | 75.9 ± 5.1 | ↑ | 53.4 ± 1.2 | 67.3 ± 3.2 | ↑ | | | | 25.2 (H)d | Hoppy (I) |
| 5,5-dimethyl-2(5H)-furanone | 62.8 ± 7.0 | 113.5 ± 7.9 | ↑ | 56.4 ± 0.2 | 79.6 ± 6.3 | ↑ | | | | | |
| 4-methyl-2-pentanone | 33.6 ± 2.9 | | ↓ | 29.0 ± 14.5 | 72.8 ± 7.1 | ↑ | | | | 60000 (A) | |
| g-nonalactone | 32.2 ± 1.1 | 107.4 ± 28.0 | ↑ | 27.8 ± 0.4 | 65.3 ± 6.5 | ↑ | 12.8 ± 3.4 | 25.5 ± 2.2 | ↑ | | |
| 4-vinylphenol | | 65.7 ± 32.9 | ↑ | | 49.1 ± 3.6 | ↓ | | 14.7 ± 3.7 | ↑ | | |
| 4-vinylguaiacol | 82.5 ± 14.5 | 306.7 ± 153.4 | ↑ | 117.6 ± 2.4 | 312.3 ± 5.4 | ↑ | 41.8 ± 8.4 | 68.8 ± 4.4 | ↑ | 21 (B)a | Clove-like, smoky(B) |
| 3-methyl-butyric acid | 55.1 ± 15.1 | 137.7 ± 8.4 | ↑ | 54.2 ± 0.7 | 72.4 ± 17.7 | ↑ | | 47.6 ± 11.1 | ↑ | 33 (F)d | Sweaty, cheese (D, B) |
| hexanoic acid | 215.9 ± 31.7 | 673.4 ± 143.4 | ↑ | 188.3 ± 2.9 | 452.3 ± 25.8 | ↑ | 75.5 ± 15.5 | 187.3 ± 53.5 | ↑ | 420 (F)d | Vegetable oil, cheese, sweaty (D, F) |
| decanoic acid | 228.5 ± 189.1 | 230.0 ± 115.0 | = | 214.9 ± 12.3 | 308.6 ± 6.9 | ↑ | 103.1 ± 13.0 | 240.5 ± 14.4 | ↑ | 1000 (F)d | Wax, tallow, rancid, soap, fatty (D, F) |
| octanoic acid | 1586.6 ± 156.3 | 2651.1 ± 102.6 | ↑ | 1595.8 ± 20.2 | 2475.0 ± 238.3 | ↑ | 740.6 ± 128.9 | 1249.0 ± 160.2 | ↑ | 500 (H)d | Vegetable oil, rancid, harsh (D, F) |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase; ↓ decrease; = constant

Table 19 - Concentration of minor volatile compounds detected in the sample C (33 cL and 75 cL bottles) and in the sample D (33 cL and 75 cL bottles) by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | C33 | | | C75 | | | D33 | | | D75 | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | 0M | 6 M | | | |
| isoamyl acetate | 1130.8 ± 12.0 | 1386.6 ± 45.9 | ↑ | 1192.1 | 1221.3 ± 162.0 | = | 755.9 ± 134.1 | 941.0 ± 99.8 | = | 975.8 | 1098.0 ± 36.1 | ↑ | 1200 (A)c | Banana (C) |
| isobutyl acetate | 59.6 ± 11.1 | 94.2 | ↑ | 52.3 | 92.0 ± 24.8 | ↑ | 25.4 ± 10.1 | 61.4 ± 5.0 | ↑ | 40.7 | 70.3 ± 8.9 | ↑ | 1600(A)c | Banana, fruity (D) |
| ethyl butyrate | 54.2 ± 3.4 | 89.7 | ↑ | 54.3 | 109.2 ± 54.6 | ↑ | 33.6 ± 6.0 | 88.9 ± 0.2 | ↑ | 38.1 | 107.6 ± 6.4 | ↑ | 20 (C)b | Papaya, sweetish, apple (B, D) |
| ethyl hexanoate | 86.8 ± 3.2 | 132.1 ± 6.2 | ↑ | 97.7 | 118.8 ± 18.0 | ↑ | 49.3 ± 5.8 | 122.0 ± 1.2 | ↑ | 62.2 | 183.7 ± 4.0 | ↑ | 210 (A)c | Fruity, green apple (D, F) |
| ethyl lactate | 143.9 ± 51.4 | 778.0 ± 287.0 | ↑ | 174.6 | 506.9 ± 83.3 | ↑ | 159.8 ± 20.2 | 443.4 ± 19.7 | ↑ | 184.1 | 391.0 ± 43.1 | ↑ | 25000 (A)c | Strawberry, raspberry, perfumed (D, F) |
| ethyl octanoate | 41.8 ± 9.9 | 43.5 ± 2.4 | = | 50.9 | 42.4 ± 2.1 | ↑ | 15.7 ± 3.4 | 34.2 ± 1.1 | ↑ | | 28.7 ± 3.5 | ↑ | 900(A)c | Apple, fruity, sweet (D,F) |
| 2-phenylethyl acetate | 368.5 ± 2.4 | 512.8 ± 25.7 | ↑ | 395.8 | 549.6 ± 274.8 | = | 306.1 ± 30.2 | 397.4 ± 34.7 | ↑ | 345.6 | 493.6 ± 1.7 | ↑ | 250 (C)b | Apple, honey, roses, flowery (D,F) |
| 2-methyl-1-propanol | 548.6 ± 126.9 | 748.5 ± 39.2 | ↑ | 626.2 | 737.6 ± 168.2 | = | 246.0 ± 53.0 | 613.8 ± 18.7 | ↑ | 421.3 | 430.2 ± 24.7 | = | - | |
| 1-hexanol | 24.5 ± 4.1 | 31.8 ± 1.7 | ↑ | 22.8 | 27.5 ± 1.3 | ↑ | 19.2 ± 1.9 | 29.1 ± 3.2 | ↑ | 21.5 | 24.1 ± 1.7 | ↑ | 8000 (C)b | |
| 2-methyl-1-butanol + 3-methyl-1-butanol | 8780.4 ± 1259.1 | 12593.7 ± 1159.4 | ↑ | 9869.1 | 11865.7 ± 1044.7 | ↑ | 5518.2 ± 1091.8 | 11662.9 ± 1494.9 | ↑ | 7659.2 | 8801.5 ± 192.6 | ↑ | - | |
| furfuryl alcohol | 133.6 ± 25.2 | 186.5 ± 35.9 | = | 154.5 | 185.5 ± 20.8 | ↑ | 47.8 ± 9.1 | 121.7 ± 9.0 | ↑ | 9.0 | 90.8 ± 8.0 | ↑ | 1000 (D)a | Moldy hay (E) |
| 2-phenylethanol | 8519.5 ± 812.7 | 13466.4 ± 10.4 | ↑ | 8882.2 | 11640.6 ± 342.6 | ↑ | 7463.2 ± 684.6 | 12303.3 ± 1394.4 | ↑ | 9273.3 | 9733.5 ± 305.2 | ↑ | | |
| methionol | 38.9 ± 19.5 | 78.0 ± 20.3 | ↑ | 52.6 | 76.4 ± 12.4 | ↑ | 28.0 ± 3.6 | 79.4 ± 6.6 | ↑ | | 54.4 ± 7.5 | ↑ | 36 (B)a | Cooked potato-like (B) |
| tryptophol | 642.1 ± 69.8 | | ↓ | 814.0 | | ↓ | 884.3 ± 85.7 | 917.0 ± 34.6 | = | 842.0 | 606.1 ± 60.8 | ↓ | | |
| linalool | 25.3 ± 12.7 | 45.8 ± 2.8 | ↑ | 27.3 | 45.0 ± 4.7 | ↑ | 32.8 ± 2.8 | 53.7 ± 3.8 | ↑ | | 53.2 ± 0.3 | ↑ | 25.2 (H)d | Hoppy (I) |
| 2-furyl methyl ketone | 47.8 ± 1.3 | 66.9 ± 8.3 | ↑ | 55.5 | 54.3 ± 0.9 | ↓ | 21.4 ± 2.2 | 39.8 ± 5.0 | ↑ | 17.5 | 35.2 ± 2.8 | ↑ | | |
| g-nonalactone | 49.1 ± 0.4 | 182.3 ± 4.6 | ↑ | 58.9 | 211.5 ± 21.0 | ↑ | 40.8 ± 3.0 | 139.2 ± 19.8 | ↑ | 39.4 | 111.8 ± 1.8 | ↑ | | |
| 4-vinylguaiacol | 87.3 ± 43.7 | 72.5 ± 36.2 | = | 96.0 | 76.9 ± 8.5 | ↓ | 65.7 ± 4.6 | 48.6 ± 2.7 | ↓ | 56.2 | 34.2 ± 17.1 | ↓ | 21 (B)a | Clove-like, smoky (B) |
| 3-methyl-butyric acid | 58.4 ± 29.2 | 131.3 ± 26.1 | ↑ | 56.6 | 129.9 ± 17.7 | ↑ | 32.0 ± 4.2 | 101.4 ± 4.9 | ↑ | | 85.8 ± 2.1 | ↑ | 33 (F)d | Sweaty, cheese (B,D) |
| hexanoic acid | 349.2 ± 64.3 | 650.5 ± 32.0 | ↑ | 352.9 | 552.2 ± 42.2 | ↑ | 208.3 ± 30.2 | 506.9 ± 54.6 | ↑ | 353.2 | 503.9 ± 26.9 | ↑ | 420 (F)d | Vegetable oil, cheese, sweaty (D,F) |
| decanoic acid | 308.8 ± 0.5 | 194.6 ± 66.0 | ↓ | 350.1 | 810.6 ± 54.6 | ↑ | 266.4 ± 55.7 | 386.5 ± 35.8 | ↑ | 207.8 | 756.9 ± 378.4 | ↑ | 1000 (F)d | Wax, tallow, rancid, soap fatty (D, F) |
| octanoic acid | 2043.4 ± 50.1 | 3040.3 ± 326.9 | ↑ | 2235.2 | 3317.5 ± 330.5 | ↑ | 1670.1 ± 201.2 | 2140.6 ± 132.8 | ↑ | 1880.4 | 2672.4 ± 91.9 | ↑ | 500 (H)d | Vegetable oil, rancid, harsh (D, F) |
| 2-methyl-propionic acid | 14.4 ± 7.2 | 47.8 ± 14.1 | ↑ | 22.2 | 51.4 ± 13.3 | ↑ | 12.1 ± 3.0 | 39.1 ± 1.0 | ↑ | | 37.1 ± 2.1 | ↑ | 200000 (C)b | Sweat, bitter cheese, rancid (D,F) |
| dimethylpyrazine | 10.0 ± 5.0 | | ↓ | 11.7 | | ↓ | | | | | | | | |
| 2-methylpiperazine | 31.9 ± 2.3 | 63.7 ± 2.2 | ↑ | 33.7 | 47.8 ± 4.5 | ↑ | | | | | | | | |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase; ↓ decrease; = constant

Vanderhaegen *et al.* (2007) listed 15 compounds as aging markers selected to represent, as much as possible, the main staling reactions (Table 20). In that study eight commercial beers (3 lager beers, 2 dark ales and 3 high-alcoholic ales) were aged for one year under normal storage conditions (20°C in the dark), and the changes with time of flavor profile and the concentration of the 15 volatile compounds were monitored.

Table 20 - Aging markers in beer and the type of aging reaction involved in their formation or degradation during storage according to Vanderhaegen *et al.* (2007)

| Aging Marker | Aging reaction |
|---|---|
| 3-Methylbutanal | Strecker degradation, oxidation of alcohol |
| 2-Isobutyl-4,5-dimethyl-1,3-dioxolane | Cyclic acetal formation of aldehyde with 2,3-butanediol |
| Furfural | Maillard reaction |
| Furfuryl ethyl ether | Etherification of ethanol and Maillard compounds |
| Diacetyl | Maillard reaction |
| Acetaldehyde | Oxidation of ethanol |
| n-Hexanal | Release of lipid oxidation products in beer |
| Iso-amyl acetate | Hydrolysis of esters produced by yeast |
| Ethyl acetate | Hydrolysis of esters produced by yeast |
| Ethyl hexanoate | Hydrolysis of esters produced by yeast |
| Ethyl lactate | Esterification of ethanol and organic acid |
| 4-Methylpentan-2-one | Degradation of hop bitter compounds |
| 3-Penten-2-one | Degradation of hop bitter compounds |
| Ethyl 2-methylbutyrate | Esterification of ethanol and organic acid |
| Ethyl 3-methylbutyrate | Esterification of ethanol and organic acid |

However, some of this compounds only showed significant results in high alcoholic beers or after six months of storage such as 3-methylbutanal, furfural, 2-furfuryl ethyl ether, diacety, ethyl 3-methylbutyrate and ethyl 2-methylbutyrate. Since 2-isobutyl-4,5-dimethyl-1,3-dioxolane is a result of a condensation reaction between 3-methylbutanal and 2,3-butanediol the 2-isobutyl-4,5-dimethyl-1,3-dioxolane is not present.

Acetaldehyde is easily formed from ethanol when oxygen is present. However, most beers are now bottled with extremely low oxygen levels (<0.2 mg/L), resulting in few oxidative aging reactions during storage. The (Vanderhaegen *et al.* 2003) study shows that the acetaldehyde only increased in bottles with air on the headspace. The craft beers used in this study were manually bottled without atmosphere control.

Both 4-methylpentan-2-one and 3-penten-2-one are formed by degradation of iso-α-acids during beer aging and this is faster at increased oxygen concentrations in the bottle. Thus, it would be expected to

find this two compounds at least for the craft beers. 3-penten-2-one has a retention time corresponding to a low resolution zone of the chromatogram, being its detection and quantification dependent on the contaminants present in the sample, thus difficult to access. 4-methylpentan-2-one was found only in pilsner beer and showed a variation for sample B75 increasing from 29.0 ± 14.5 μg/L to 72.8 ± 7.1 μg/L (Table 18) however these values are far below the threshold level (60000 μg/L).

n-Hexanal is a product of lipid oxidation and can be formed during beer production. Aldehydes originating from lipid degradation can be released during beer storage from adducts with amino acids or proteins. Differences in lipid oxidation during beer production and aldehyde release during storage or reactions of aldehydes with alcohols, water or sulfite, producing acetals, enols or sulfite adducts, may affect their concentration and explain the fact that this compound has not been found in the analyzed beers (Vanderhaegen *et al.* 2007).

In fact none aldehyde or Maillard reaction products were found in the analyzed beers (Annexes) which can be seen as a good result since some of these compounds are responsible for off-flavors such as diacetyl (buttery), methional (cooked potatoes, worty) or (E)-2-nonenal (cardboard, papery, cucumber). However others are responsible for pleasant flavors as the acetaldehyde (green apple, fruity), phenyl acetaldehyde (hyacinth, flowery, roses) or 3-methylbutanal (malty, cherry, almond, chocolate).

Esters are produced by yeast during fermentation and give pleasant fruity flavors to beer. Isoamyl acetate is produced by yeast however, during storage, the concentration of this ester can decrease due to hydrolysis. This fact can be seen for some of the craft beers as for sample A (Table 17), inclusively for 75 cL corked bottle the concentration of this compound decreases (from 1965.7 ± 28.7 μg/L to 765.5 ± 35.3 μg/L) below threshold level (1200 μg/L), and for sample B (Table 18) (however it was already below the threshold). For samples C33 and D (Table 19) a slight increase is observed which can be explained by the fact that the craft beers contain the yeast in bottle. For commercial ale (Table 17) there is a large increase (from 2223.7 μg/L to 3180.2 ± 88.9 μg/L) which may indicate that there may have been yeast lysis. This beer style is pasteurized and depending on the storage temperature the lysis may occur due to the high pressures of this style. Despite of being present in a much lower concentration ethyl hexanoate showed a similar behavior but this is below the thresholds for all the beers.

Certain volatile esters (ethyl 3-methylbutyrate, ethyl 2-methyl-butyrate, ethyl 2-methylpropionate, ethyl nicotinate, diethyl succinate, ethyl lactate, ethyl phenylacetate, ethyl formate, ethyl furoate and ethyl cinnamate) are synthesized during beer aging (Vanderhaegen *et al.* 2006). Ethyl lactate results from an esterification reaction between ethanol and an organic acid (e.g., lactic acid). This can be seen for all the

craft beers that, despite showing different concentrations, all present a significant increase of this compound. Diethyl succinate has the same behavior however both this compounds are too far from the threshold level (1200).

Although not all the ageing markers described for (Vanderhaegen *et al.* 2007) have been found in the analyzed beers, other important compounds were found like higher alcohols, ketones and aliphatic acids.

Besides ethanol, beer contains several alcohols which are derived mainly from yeast metabolism and from hops and malts. These compounds are so-called higher alcohols as they have a larger molecular weight. Associated to this group is a reaction, the oxidation of higher alcohols forming the corresponding aldehydes. However, as previously said, none aldehyde has found. All alcohols concentrations are below the threshold level except methionol (36 μg/L) that increased in all beers. However it is important to note that this threshold refers to olfactory perception threshold in water which is different from the perception in beer.

5,5-dimethyl-2(5H)-furanone is a ketone which is associated with degradation of hop compounds and is supposed to increase as it can be seen in the Table 18. As the threshold of this compound was not found in literature, it cannot be said that the increase of concentration is significant. According to Vanderhaegen *et al.* (2006) lactones such as γ-nonalactone (peach, fruity) tend to increase in concentration as happens in beers studied as for the 5,5-dimethyl-2(5H)-furanone, but the threshold was not found.

The degradation of the carbonyl side-chain of alpha-acids and beta-acids releases 2-methyl-propionic acid and 3- methyl-butyric acid. It is expected an increase in the concentration of this compounds as can be seen in the Table 17, 18 and 19 but only the 3-methyl-butyric acid is above the threshold level.

Most of the acids are of yeast origin or, more precisely, they are yeast waste products. The aliphatic acids with short to middle carbon chain length have distinctive and familiar odors; hexanoic, octanoic and decanoic acids can be responsible for off-flavors in beer described as fatty acids, vegetable oil, rancid and cheesy. The hexanoic and octanoic acids increase to above the threshold level (420 and 500 μg/L respectively) however, the aromas associated to these acids weren't detected on the sensorial analysis.

# 7. CONCLUSIONS/RECOMMENDATIONS

The main objective of this work was to study the changes that occur in beer during six months of storage. Six types of beers were studied: four craft beers and two commercial beers. It was demonstrated that:

Craft beers have flavor/aroma more intense than commercial beers;

Overall, beer's sensorial characteristics remained stable during the six months of storage, as demonstrated by sensorial analysis;

Some changes were observed in minor compounds analysis, which were not reflected in the sensorial analysis as for example:

- Increase of hexanoic and decanoic acids above the threshold level (420 and 500 μg/L respectively);
- Increase of methionol (cooked potato aroma) above the threshold level (36 μg/L);
- Different behavior of the same compounds for each beer as for example: decrease of isoamyl acetate for samples A and B and increase for samples C and D.

The results of minor compound analysis were in line with the aromatic profiles obtained by sensory analysis as well as those portrayed in the literature:

- Weiss – characterized by fruity (presence of isoamyl acetate) and phenolic aromas (4-vinylguaicol);
- Pilsner – characterized by hoppy aroma and taste (linalool in high concentration);
- Stout – caramelized/roasted intense aroma (pyrazines);
- Red Ale – identical to Pilsner but the aroma from the hops is of medium intensity (higher concentration of linalool than Stout and Weiss but smaller than Pilsner) which allows to identify the sweet and caramel flavor also of medium intensity characteristic of this recipe.

Chemical analysis showed that the craft beers have:

- Higher concentrations of organic acids than commercial beers except for citric acid;
- The yield of the fermentation is lower when compared to industrial strains - there is a level of some fermentable sugars whiting the storage time (samples B, C and D).

In general, the main objectives of this dissertation have been achieved, however some work can be done in order to understand at which point the maturation of the craft beer stops and the deterioration starts. For this, the study should continue over more time (maybe over a year) and more analysis of minor compounds should be made (for example at the third, sixth, ninth and twelfth month instead of initial and final).

The validation of the extraction method for minor compounds (for subsequent analysis by GC-MS). This work showed that:

- The correlation coefficient for the calibration curves varied between 0.962 and 0.998 and the sensibility between 0.02 and 1.242. These large ranges were observed because these parameters depend on both extraction efficiency and detector response for each compound;
- The LD and LQ also showed wide ranges, which may be related to differences in chemical and physical proprieties of each compound;
- The accuracy of the method was verified by repeatability and intermediate precision;
- The tests for matrix effect, contact time and the spiking test appear to show that the ethyl octanoate and the ethyl decanoate evaporated from the stock solution. Most of the other compounds showed results between the expected.

The results show that the validation of the extraction method was a success since the method satisfies the determined specifications for each validation parameter.

For improvement of this work some recommendations can be advanced: more tests should be done as ruggedness and robustness, specificity and selectivity in order to have a more complete validation. It also can be important to do the spiking test in different test materials (wine, vinegar). All tests should be in the shortest period of time in order to prevent the evaporation of the stock solution compounds.

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# ANNEXES

Table A 1 - Concentration of minor volatile compounds detected in the sample A (33 cL and 75 cL bottles) and in the commercial Ale by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | A33 | | | A75 | | | Commercial Ale | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | | |
| isoamyl acetate | 1853.3 ± 57.9 | 1247.2 ± 42.7 | ↓ | 1965.7 ± 28.7 | 765.5 ± 35.3 | ↓ | 2223.7 | 3180.2 ± 88.9 | ↑ | 1200 (A)c | Banana (C) |
| isobutyl acetate | 161.9 ± 14.5 | 181.1 ± 4.3 | = | 166.2 ± 4.5 | 131.5 ± 13.1 | ↓ | 177.2 | 237.5 ± 0.5 | ↑ | 1600(A)c | Banana, fruity (D) |
| ethyl butyrate | 49.1 ± 1.3 | 69.7 ± 1.0 | ↑ | | 54.6 ± 4.4 | ↑ | 64.3 | 108.4 ± 0.6 | ↑ | 20 (C)b | Papaya, butter, sweetish, apple (B, D) |
| ethyl hexanoate | 97.3 ± 7.9 | 74.4 ± 5.4 | ↓ | 84.5 ± 1.5 | 84.1 ± 7.6 | = | 38.7 | 92.6 ± 1.7 | ↑ | 210 (A)c | Fruity, green apple (D, F) |
| ethyl lactate | 365.1 ± 130.2 | 844.5 ± 133.7 | ↑ | 436.0 ± 11.4 | 721.2 ± 56.0 | ↑ | 23.4 | 30.7 ± 0.6 | ↑ | 25000 (A)c | Strawberry, raspberry, perfumed (D, F) |
| ethyl octanoate | 120.9 ± 23.3 | 38.4 ± 1.4 | ↓ | 113.8 ± 4.1 | 86.7 ± 5.4 | ↓ | | 15.4 ± 0.2 | ↑ | 900(A)c | Apple, fruity (F); sweet (D) |
| furfuryl acetate | 5.7 ± 0.8 | | ↓ | | | | | | | | |
| ethyl decanoate | | | | 15.5 ± 3.7 | | ↓ | | | | 570(A)c | |
| diethyl succinate | 4.6 ± 0.8 | 29.8 ± 6.9 | ↑ | 4.1 ± 0.2 | 39.0 ± 0.5 | ↑ | | | | 1200(A)c | |
| 2-phenylethyl acetate | 458.4 ± 1.3 | 439.4 ± 18.0 | = | 456.6 ± 15.5 | 318.0 ± 18.7 | ↓ | 789.9 | 881.1 ± 8.4 | ↑ | 250 (C)b | Apple, honey, roses, flowery (D) (F) |
| ethyl hexadecanoate | | | | | | | | | | | |
| 2-methyl-1-propanol | 629.6 ± 10.2 | 1368.6 ± 265.3 | ↑ | 762.6 ± 3.8 | 1001.7 ± 215.2 | ↑ | 551.7 | 563.7 ± 31.1 | = | - | |
| 3-methyl-2-buten-1-ol | 9.0 ± 1.6 | | ↓ | 6.9 ± 0.4 | | ↓ | 9.0 | | ↓ | - | |
| 1-hexanol | 20.9 ± 1.0 | 31.4 ± 3.5 | ↑ | 21.7 ± 1.5 | 27.5 ± 2.6 | ↑ | | 8.3 ± 0.7 | ↑ | 8000 (C)b | |
| 3-etoxi-1-propanol | 6.4 ± 0.1 | 14.1 ± 2.6 | ↑ | 6.9 ± 0.4 | 11.2 ± 2.8 | ↑ | | | | - | |
| Z-3-hexenol | | | | | | | | | | - | |
| 2-methyl-1-butanol + 3-methyl-1-butanol | 907.5 ± 5.0 | 17561.9 ± 2856.7 | ↑ | 1032.5 ± 0.5 | 12320.9 ± 2076.4 | ↑ | 8422.9 | 9709.5 ± 652.2 | ↑ | - | |
| 1-octen-3-ol | | | | | | | | | | - | |
| 1-heptanol | | | | | | | | | | - | Coconut, ketonic solvent, unpleasant (F) |
| 1-octanol | 6.1 ± 0.1 | | ↓ | | | | | | | 900 (F)c | Coconut, walnut, oily (F) |
| 1,3-propanediol | 9.1 ± 0.2 | | ↓ | 7.4 ± 0.8 | | ↓ | 9.8 | 7.6 ± 0.3 | ↓ | | |
| furfuryl alcohol | 37.7 ± 1.9 | 94.0 ± 17.1 | ↑ | 40.1 ± 2.3 | 74.2 ± 7.9 | ↑ | 40.7 | 54.0 ± 1.2 | ↑ | 1000 (D)a | Moldy hay (E) |
| 2-phenylethanol | 5806.9 ± 174.6 | 9986.8 ± 1851.5 | ↑ | 5744.4 ± 179.5 | 7599.8 ± 875.3 | ↑ | 5769.5 | 5668.6 ± 70.7 | ↓ | | |
| 1-butanol | | | | 10.4 ± 0.2 | | ↓ | | | | 590 (B)a | Malty, solvent-like (B); fusel (D) |
| methionol | 151.6 ± 4.5 | 320.2 ± 55.0 | ↑ | 169.0 ± 0.3 | 259.5 ± 27.5 | ↑ | 172.7 | 193.1 ± 3.4 | ↑ | 36 (B)a | Cooked potato-like (B) |
| tyrosol | 8.7 ± 1.6 | | ↓ | 12.5 ± 1.9 | | ↓ | 18.4 | | ↓ | - | Bitter, chemical (F) |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase ; ↓ decrease; = constant

Table A 1 – (Continued) Concentration of minor volatile compounds detected in sample A (33 cL and 75 cL bottles) and in the commercial Ale by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | A33 | | | A75 | | | Commercial Ale | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | | |
| tryptophol | 554.6 ± 1.3 | 430.4 ± 10.2 | ↓ | 469.0 ± 56.2 | 478.5 ± 52.3 | = | 761.9 | 528.3 ± 20.0 | ↓ | | |
| linalool | 4.1 ± 1.0 | | ↓ | 4.2 ± 0.5 | | ↓ | 2.1 | | ↓ | 25.2 (H)d | Hoppy (I) |
| 2-methyltetrahydrofuran-3-one | 9.5 ± 0.3 | | ↓ | 9.4 ± 1.0 | | ↓ | | 7.0 ± 0.7 | ↑ | | |
| 2-furyl methyl ketone | | | | | | | 9.9 | 15.0 ± 0.1 | ↑ | | |
| 5,5-dimethyl-2(5H)-furanone | | | | | | | | 41.5 ± 1.3 | ↑ | | |
| 6-methyl-5-hepten-2-one | | | | | | | | | | | |
| 3-hydroxy-2-butanone | 56.7 ± 6.8 | | ↓ | 39.5 ± 1.2 | | ↓ | | | | | |
| 4-methyl-2-pentanone | | | | | | | | | | 60000 (A) | |
| g-nonalactone | 40.2 ± 0.3 | 170.6 ± 0.4 | ↑ | 48.0 ± 4.7 | 164.9 ± 20.3 | ↑ | 32.4 | 46.3 ± 0.4 | ↑ | | |
| g-capralactone | | | | | | | 9.6 | | ↓ | | |
| 4-ethylguaiacol | 8.0 ± 1.0 | | ↓ | 10.2 ± 2.0 | | ↓ | 38.0 | 42.2 ± 0.5 | ↑ | | |
| 4-vinylphenol | 34.7 ± 24.3 | | ↓ | 8.3 ± 1.5 | | ↓ | 182.5 | 167.6 ± 2.5 | ↓ | | |
| 4-ethylphenol | 328.2 ± 70.3 | 548.5 ± 41.3 | ↑ | 420.1 ± 14.0 | 496.9 ± 3.5 | ↑ | | | | | |
| 4-vinylguaiacol | 1492.2 ± 16.1 | 1100.7 ± 120.4 | ↑ | 1835.6± 86.6 | 979.6 ± 39.6 | ↓ | 1666.5 | 1209.7 ± 16.6 | ↓ | 21 (B)a | Clove-like, smoky(B) |
| 3-methyl-butyric acid | 37.3 ± 0.5 | 102.6 ± 10.1 | ↑ | 48.4 ± 5.6 | 86.8 ± 4.6 | ↑ | 35.2 | 44.4 ± 0.5 | ↑ | 33 (F)d | Sweaty (B); cheese (D) |
| hexanoic acid | 214.3 ± 6.5 | 584.2 ± 91.6 | ↑ | 238.5 ± 18.7 | 471.1 ± 57.2 | ↑ | 273.7 | 389.6 ± 14.4 | ↑ | 420 (F)d | Vegetable oil (F); cheese, sweaty (D) |
| decanoic acid | 25.4 ± 7.5 | | ↓ | 64.7 ± 16.4 | | ↓ | 802.0 | 1380.0 ± 65.9 | ↑ | 1000 (F)d | Wax, tallow, rancid, soap (F) fatty (D) |
| octanoic acid | 1249.1 ± 76.7 | 2176.2 ± 88.6 | ↑ | 1483.7 ± 56.9 | 2116.8 ± 25.8 | ↑ | 2047.8 | 2765.8 ± 5.7 | ↑ | 500 (H)d | Vegetable oil (F); rancid, harsh (D) |
| 2-methyl-propionic acid | 15.1 ± 2.0 | 62.4 ± 1.4 | ↑ | 17.7 ± 6.8 | 64.5 ± 1.3 | ↑ | 12.1 | 14.6 ± 1.2 | ↑ | 200000 (C)b | Sweat, bitter (F); cheese, rancid (D) |
| dimethylpyrazine | | | | | | | | | | | |
| 2-methylpiperazine | | | | | | | | | | | |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase ; ↓ decrease; = constant

Table A 2 - Concentration of minor volatile compounds detected in the sample B (33 cL and 75 cL bottles) and in the commercial Lager by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | B33 | | | B75 | | | Commercial Lager | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0M | 6 M | | | |
| isoamyl acetate | 767.3 ± 40.3 | 453.0 ± 7.5 | ↓ | 710.7 ± 3.8 | 455.1 ± 21.4 | ↓ | 619.6 ± 89.3 | 784.2 ± 56.2 | ↑ | 1200 (A)c | Banana (C) |
| isobutyl acetate | 37.9 | | ↓ | 22.9 ± 5.5 | | ↓ | | 15.8 ± 1.3 | ↑ | 1600(A)c | Banana, fruity (D) |
| ethyl butyrate | 27.5 ± 2.2 | 78.0 ± 9.9 | ↑ | | 65.4 ± 6.0 | ↑ | 36.2 ± 4.9 | 56.0 ± 5.4 | ↑ | 20 (C)b | Papaya, butter, sweetish, apple (B, D) |
| ethyl hexanoate | 144.9 ± 22.8 | 109.3 ± 15.5 | ↓ | 104.0 ± 0.5 | 85.5 ± 3.8 | ↓ | 30.4 ± 2.4 | 42.5 ± 3.6 | ↑ | 210 (A)c | Fruity, green apple (D, F) |
| ethyl lactate | 17.0 ± 4.6 | 81.0 ± 23.5 | ↑ | 8.6 ± 0.5 | 28.6 ± 0.1 | ↑ | | | | 25000 (A)c | Strawberry, raspberry, perfumed (D, F) |
| ethyl octanoate | 242.3 ± 26.3 | 129.1 ± 39.2 | ↓ | 230.5 ± 1.5 | 81.7 ± 3.8 | ↓ | | | | 900(A)c | Apple, fruity (F); sweet (D) |
| furfuryl acetate | | | | | | | | | | | |
| ethyl decanoate | 76.6 ± 42.1 | 18.5 ± 2.9 | ↓ | 46.4 ± 0.3 | 12.6 ± 1.5 | ↓ | | | | 570(A)c | |
| diethyl succinate | 16.4 ± 5.9 | 47.3 ± 8.2 | ↑ | 12.7 ± 0.9 | 31.4 ± 3.6 | ↑ | | | | 1200(A)c | |
| 2-phenylethyl acetate | 377.4 ± 14.4 | 251.2 ± 2.1 | ↓ | 390.5 ± 9.8 | 256.4 ± 20.6 | ↓ | 257.2 ± 43.2 | 380.7 ± 30.6 | ↑ | 250 (C)b | Apple, honey, roses, flowery (D) (F) |
| ethyl hexadecanoate | 17.5 ± 4.5 | | ↓ | 24.5 ± 0.2 | | ↓ | | | | | |
| 2-methyl-1-propanol | 226.0 ± 53.8 | 482.2 ± 85.3 | ↑ | 173.9 ± 0.4 | 287.5 ± 20.2 | ↑ | 134.8 ± 22.3 | 326.8 ± 98.1 | ↑ | - | |
| 3-methyl-2-buten-1-ol | 15.7 ± 2.2 | | ↓ | 14.5 ± 0.9 | | ↓ | 7.0 ± 0.0 | | ↓ | - | |
| 1-hexanol | 16.3 ± 2.5 | 26.7 ± 2.8 | ↑ | 11.9 ± 0.0 | 16.4 ± 1.4 | ↑ | | | | 8000 (C)b | |
| 3-etoxi-1-propanol | | | | | | | | | | - | |
| Z-3-hexenol | 6.8 ± 1.6 | 14.6 ± 2.3 | ↑ | 4.7 ± 0.1 | 9.4 ± 0.1 | ↑ | | | | - | |
| 2-methyl-1-butanol + 3-methyl-1-butanol | 7036.7 ± 1877.7 | 14049.3 ± 2161.5 | ↑ | 5653.2 ± 89.1 | 8570.4 ± 715.8 | ↑ | 4149.7 ± 684.7 | 8936.9 ± 2408.4 | ↑ | - | |
| 1-octen-3-ol | 13.4 ± 4.6 | | ↓ | 7.9 ± 0.0 | | ↓ | | | | - | |
| 1-heptanol | 9.7 ± 2.0 | | ↓ | 6.2 ± 0.2 | | ↓ | | | | - | Coconut, ketonic solvent, unpleasant (F) |
| 1-octanol | 31.9 ± 4.6 | 28.0 ± 0.4 | = | 25.9 ± 1.0 | 26.5 ± 1.5 | = | | 6.8 ± 1.0 | ↑ | 900 (F)c | Coconut, walnut, oily (F) |
| 1,3-propanediol | | | | | | | | | | | |
| furfuryl alcohol | 57.6 ± 11.9 | 120.7 ± 9.3 | ↑ | 40.4 ± 0.0 | 73.4 ± 6.7 | ↑ | 27.0 ± 4.7 | 33.9 ± 7.4 | ↑ | 1000 (D)a | Moldy hay (E) |
| 2-phenylethanol | 7016.8 ± 765.1 | 13262.0 ± 844.6 | ↑ | 6201.1 ± 134.4 | 8492.1 ± 867.3 | ↑ | 4405.8 ± 584.2 | 7175.8 ± 1589.1 | ↑ | | |
| 1-butanol | 16.9 ± 5.3 | | ↓ | 13.4 ± 0.5 | | ↓ | 13.6 ± 2.4 | | ↓ | 590 (B)a | Malty, solvent-like (B); fusel (D) |
| methionol | 24.5 ± 7.0 | 76.8 ± 6.4 | ↑ | 20.0 ± 0.2 | 48.4 ± 2.1 | ↑ | 24.8 ± 3.8 | 57.6 ± 18.9 | ↑ | 36 (B)a | Cooked potato-like (B) |
| tyrosol | 48.4 ± 17.9 | | ↓ | 43.9 ± 2.8 | | ↓ | 16.2 ± 9.7 | | ↓ | - | Bitter, chemical (F) |
| tryptophol | 57.3 ± 24.2 | | ↓ | 54.0 ± 4.6 | | ↓ | 41.5 ± 5.4 | | ↓ | | |
| linalool | 57.5 ± 0.9 | 75.9 ± 5.1 | ↑ | 53.4 ± 1.2 | 67.3 ± 3.2 | ↑ | | | | 25.2 (H)d | Hoppy (I) |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase ; ↓ decrease; = constant

Table A 2 – (Continued) Concentration of minor volatile compounds detected in the sample B (33 cL and 75 cL bottles) and in the commercial Lager by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | B33 | | | B75 | | | Commercial Lager | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | | |
| 2-methyltetrahydrofuran-3-one | | | | | | | | | | | |
| 2-furyl methyl ketone | | | | | | | | 8.1 ± 1.6 | ↑ | | |
| 5,5-dimethyl-2(5H)-furanone | 62.8 ± 7.0 | 113.5 ± 7.9 | ↑ | 56.4 ± 0.2 | 79.6 ± 6.3 | ↑ | | | | | |
| 6-methyl-5-hepten-2-one | 12.0 ± 3.1 | 8.7 ± 4.3 | ↓ | 9.2 ± 0.5 | 9.0 ± 0.5 | ↓ | | | | | |
| 3-hydroxy-2-butanone | 12.6 ± 4.6 | | ↓ | | | | | | | | |
| 4-methyl-2-pentanone | 33.6 ± 2.9 | | ↓ | 29.0 ± 14.5 | 72.8 ± 7.1 | ↑ | | | | 60000 (A) | |
| g-nonalactone | 32.2 ± 1.1 | 107.4 ± 28.0 | ↑ | 27.8 ± 0.4 | 65.3 ± 6.5 | ↑ | 12.8 ± 3.4 | 25.5 ± 2.2 | ↑ | | |
| g-capralactone | | | | | | | | | | | |
| 4-ethylguaiacol | | | | | | | | | | | |
| 4-vinylphenol | | 65.7 ± 32.9 | ↑ | | 49.1 ± 3.6 | ↓ | | 14.7 ± 3.7 | ↑ | | |
| 4-ethylphenol | | | | | | | | | | | |
| 4-vinylguaiacol | 82.5 ± 14.5 | 306.7 ± 153.4 | ↑ | 117.6 ± 2.4 | 312.3 ± 5.4 | ↑ | 41.8 ± 8.4 | 68.8 ± 4.4 | ↑ | 21 (B)a | Clove-like, smoky(B) |
| 3-methyl-butyric acid | 55.1 ± 15.1 | 137.7 ± 8.4 | ↑ | 54.2 ± 0.7 | 72.4 ± 17.7 | ↑ | | 47.6 ± 11.1 | ↑ | 33 (F)d | Sweaty (B); cheese (D) |
| hexanoic acid | 215.9 ± 31.7 | 673.4 ± 143.4 | ↑ | 188.3 ± 2.9 | 452.3 ± 25.8 | ↑ | 75.5 ± 15.5 | 187.3 ± 53.5 | ↑ | 420 (F)d | Vegetable oil (F); cheese, sweaty (D) |
| decanoic acid | 228.5 ± 189.1 | 230.0 ± 115.0 | = | 214.9 ± 12.3 | 308.6 ± 6.9 | ↑ | 103.1 ± 13.0 | 240.5 ± 14.4 | ↑ | 1000 (F)d | Wax, tallow, rancid, soap (F) fatty (D) |
| octanoic acid | 1586.6 ± 156.3 | 2651.1 ± 102.6 | ↑ | 1595.8 ± 20.2 | 2475.0 ± 238.3 | ↑ | 740.6 ± 128.9 | 1249.0 ± 160.2 | ↑ | 500 (H)d | Vegetable oil (F); rancid, harsh (D) |
| 2-methyl-propionic acid | 5.9 ± 4.3 | 27.5 ± 6.8 | ↑ | 8.6 ± 0.3 | 17.6 ± 0.6 | ↑ | | | | 200000 (C)b | Sweat, bitter (F); cheese, rancid (D) |
| dimethylpyrazine | | | | | | | | | | | |
| 2-methylpiperazine | | | | | | | | | | | |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water; b Olfactory perception threshold in hydro-alcoholic solution; c Olfactory difference threshold in beer; d Olfactory threshold in model wine.

↑ increase ; ↓ decrease; = constant

Table A 3 - Concentration of minor volatile compounds detected in the sample C (33 cL and 75 cL bottles) and in the sample D (33 cL and 75 cL bottles) by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | C33 | | | C75 | | | D33 | | | D75 | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | 0M | 6 M | | | |
| isoamyl acetate | 1130.8 ± 12.0 | 1386.6 ± 45.9 | ↑ | 1192.1 | 1221.3 ± 162.0 | = | 755.9 ± 134.1 | 941.0 ± 99.8 | = | 975.8 | 1098.0 ± 36.1 | ↑ | 1200 (A)c | Banana (C) |
| isobutyl acetate | 59.6 ± 11.1 | 94.2 | ↑ | 52.3 | 92.0 ± 24.8 | ↑ | 25.4 ± 10.1 | 61.4 ± 5.0 | ↑ | 40.7 | 70.3 ± 8.9 | ↑ | 1600(A)c | Banana, fruity (D) |
| ethyl butyrate | 54.2 ± 3.4 | 89.7 | ↑ | 54.3 | 109.2 ± 54.6 | ↑ | 33.6 ± 6.0 | 88.9 ± 0.2 | ↑ | 38.1 | 107.6 ± 6.4 | ↑ | 20 (C)b | Papaya, butter, sweetish, apple (B, D) |
| ethyl hexanoate | 86.8 ± 3.2 | 132.1 ± 6.2 | ↑ | 97.7 | 118.8 ± 18.0 | ↑ | 49.3 ± 5.8 | 122.0 ± 1.2 | ↑ | 62.2 | 183.7 ± 4.0 | ↑ | 210 (A)c | Fruity, green apple (D, F) |
| ethyl lactate | 143.9 ± 51.4 | 778.0 ± 287.0 | ↑ | 174.6 | 506.9 ± 83.3 | ↑ | 159.8 ± 20.2 | 443.4 ± 19.7 | ↑ | 184.1 | 391.0 ± 43.1 | ↑ | 25000 (A)c | Strawberry, raspberry, perfumed (D, F) |
| ethyl octanoate | 41.8 ± 9.9 | 43.5 ± 2.4 | = | 50.9 | 42.4 ± 2.1 | ↑ | 15.7 ± 3.4 | 34.2 ± 1.1 | ↑ | | 28.7 ± 3.5 | ↑ | 900(A)c | Apple, fruity (F); sweet (D) |
| furfuryl acetate | 14.6 ± 7.3 | | ↓ | 14.5 | | ↓ | 6.3 ± 0.1 | | ↓ | | | | | |
| ethyl decanoate | | | | | | | | | | | | | 570(A)c | |
| diethyl succinate | | 23.9 ± 3.9 | ↑ | | 23.5 ± 1.6 | ↑ | | 19.0 ± 6.8 | ↑ | | 16.3 ± 0.6 | ↑ | 1200(A)c | |
| 2-phenylethyl acetate | 368.5 ± 2.4 | 512.8 ± 25.7 | ↑ | 395.8 | 549.6 ± 274.8 | = | 306.1 ± 30.2 | 397.4 ± 34.7 | ↑ | 345.6 | 493.6 ± 1.7 | ↑ | 250 (C)b | Apple, honey, roses, flowery (D) (F) |
| ethyl hexadecanoate | | | | | | | | | | | | | | |
| 2-methyl-1-propanol | 548.6 ± 126.9 | 748.5 ± 39.2 | ↑ | 626.2 | 737.6 ± 168.2 | = | 246.0 ± 53.0 | 613.8 ± 18.7 | ↑ | 421.3 | 430.2 ± 24.7 | = | - | |
| 3-methyl-2-buten-1-ol | 16.6 ± 8.3 | | ↓ | 18.8 | | | 15.3 ± 0.9 | | ↓ | | | | - | |
| 1-hexanol | 24.5 ± 4.1 | 31.8 ± 1.7 | ↑ | 22.8 | 27.5 ± 1.3 | ↑ | 19.2 ± 1.9 | 29.1 ± 3.2 | ↑ | 21.5 | 24.1 ± 1.7 | ↑ | 8000 (C)b | |
| 3-etoxi-1-propanol | 18.3 ± 4.8 | | ↓ | 20.6 | | ↓ | 8.9 ± 1.6 | 14.9 ± 0.3 | ↑ | 14.4 | 11.9 ± 0.9 | ↓ | - | |
| Z-3-hexenol | | | | | | | | | | | | | - | |
| 2-methyl-1-butanol + 3-methyl-1-butanol | 8780.4 ± 1259.1 | 12593.7 ± 1159.4 | ↑ | 9869.1 | 11865.7 ± 1044.7 | ↑ | 5518.2 ± 1091.8 | 11662.9 ± 1494.9 | ↑ | 7659.2 | 8801.5 ± 192.6 | ↑ | - | |
| 1-octen-3-ol | | | | | | | | | | | | | - | |
| 1-heptanol | | | | | | | | | | | | | - | Coconut, ketonic solvent, unpleasant (F) |
| 1-octanol | 11.9 ± 5.9 | | ↓ | 12.5 | | ↓ | | 13.7 ± 1.4 | ↑ | | 13.2 ± 1.4 | ↑ | 900 (F)c | Coconut, walnut, oily (F) |
| 1,3-propanediol | | | | | | | | | | | | | | |
| furfuryl alcohol | 133.6 ± 25.2 | 186.5 ± 35.9 | = | 154.5 | 185.5 ± 20.8 | ↑ | 47.8 ± 9.1 | 121.7 ± 9.0 | ↑ | 9.0 | 90.8 ± 8.0 | ↑ | 1000 (D)a | Moldy hay (E) |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water, b Olfactory perception threshold in hydro-alcoholic solution, c Olfactory difference threshold in beer, d Olfactory threshold in model wine

↑ increase , ↓ decrease, = constant

Table A 3 – (Continued) Concentration of minor volatile compounds detected in the sample C (33 cL and 75 cL bottles) and in the sample D (33 cL and 75 cL bottles) by GC-MS at initial time (0 M) and after 6 months of storage (6 M); odor threshold and descriptors reported in literature

| | C33 | | | C75 | | | D33 | | | D75 | | | Threshold level (µg/L) | Flavor descriptors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | 0 M | 6 M | | | |
| 2-phenylethanol | 8519.5 ± 812.7 | 13466.4 ± 10.4 | ↑ | 8882.2 | 11640.6 ± 342.6 | ↑ | 7463.2 ± 684.6 | 12303.3 ± 1394.4 | ↑ | 9273.3 | 9733.5 ± 305.2 | ↑ | | |
| 1-butanol | | | | | | | | | | | | | 590 (B)a | Malty, solvent-like (B); fusel (D) |
| methionol | 38.9 ± 19.5 | 78.0 ± 20.3 | ↑ | 52.6 | 76.4 ± 12.4 | ↑ | 28.0 ± 3.6 | 79.4 ± 6.6 | ↑ | | 54.4 ± 7.5 | ↑ | 36 (B)a | Cooked potato-like (B) |
| tyrosol | 73.7 ± 17.9 | | ↓ | | | | 74.5 ± 13.8 | | ↓ | 81.1 | | ↓ | - | Bitter, chemical (F) |
| tryptophol | 642.1 ± 69.8 | | ↓ | 814.0 | | ↓ | 884.3 ± 85.7 | 917.0 ± 34.6 | = | 842.0 | 606.1 ± 60.8 | ↓ | | |
| linalool | 25.3 ± 12.7 | 45.8 ± 2.8 | ↑ | 27.3 | 45.0 ± 4.7 | ↑ | 32.8 ± 2.8 | 53.7 ± 3.8 | ↑ | | 53.2 ± 0.3 | ↑ | 25.2 (H)d | Hoppy (I) |
| 2-furyl methyl ketone | 47.8 ± 1.3 | 66.9 ± 8.3 | ↑ | 55.5 | 54.3 ± 0.9 | ↓ | 21.4 ± 2.2 | 39.8 ± 5.0 | ↑ | 17.5 | 35.2 ± 2.8 | ↑ | | |
| 5,5-dimethyl-2(5H)-furanone | | | | | | | | | | | | | | |
| 3-hydroxy-2-butanone | | | | | | | 40.0 ± 8.8 | | ↓ | 7.0 | | ↓ | | |
| 4-methyl-2-pentanone | | | | | | | | | | | | | 60000 (A) | |
| g-nonalactone | 49.1 ± 0.4 | 182.3 ± 4.6 | ↑ | 58.9 | 211.5 ± 21.0 | ↑ | 40.8 ± 3.0 | 139.2 ± 19.8 | ↑ | 39.4 | 111.8 ± 1.8 | ↑ | | |
| g-capralactone | | | | | | | | 45.4 ± 6.1 | ↑ | | 42.8 ± 4.5 | ↑ | | |
| 4-ethylguaiacol | | | | | | | | | | | | | | |
| 4-vinylphenol | | | | | | | | | | | | | | |
| 4-ethylphenol | | | | | | | | | | | | | | |
| 4-vinylguaiacol | 87.3 ± 43.7 | 72.5 ± 36.2 | = | 96.0 | 76.9 ± 8.5 | ↓ | 65.7 ± 4.6 | 48.6 ± 2.7 | ↓ | 56.2 | 34.2 ± 17.1 | ↓ | 21 (B)a | Clove-like, smoky(B) |
| 3-methyl-butyric acid | 58.4 ± 29.2 | 131.3 ± 26.1 | ↑ | 56.6 | 129.9 ± 17.7 | ↑ | 32.0 ± 4.2 | 101.4 ± 4.9 | ↑ | | 85.8 ± 2.1 | ↑ | 33 (F)d | Sweaty (B); cheese (D) |
| hexanoic acid | 349.2 ± 64.3 | 650.5 ± 32.0 | ↑ | 352.9 | 552.2 ± 42.2 | ↑ | 208.3 ± 30.2 | 506.9 ± 54.6 | ↑ | 353.2 | 503.9 ± 26.9 | ↑ | 420 (F)d | Vegetable oil (F); cheese, sweaty (D) |
| decanoic acid | 308.8 ± 0.5 | 194.6 ± 66.0 | ↓ | 350.1 | 810.6 ± 54.6 | ↑ | 266.4 ± 55.7 | 386.5 ± 35.8 | ↑ | 207.8 | 756.9 ± 378.4 | ↑ | 1000 (F)d | Wax, tallow, rancid, soap (F) fatty (D) |
| octanoic acid | 2043.4 ± 50.1 | 3040.3 ± 326.9 | ↑ | 2235.2 | 3317.5 ± 330.5 | ↑ | 1670.1 ± 201.2 | 2140.6 ± 132.8 | ↑ | 1880.4 | 2672.4 ± 91.9 | ↑ | 500 (H)d | Vegetable oil (F); rancid, harsh (D) |
| 2-methyl-propionic acid | 14.4 ± 7.2 | 47.8 ± 14.1 | ↑ | 22.2 | 51.4 ± 13.3 | ↑ | 12.1 ± 3.0 | 39.1 ± 1.0 | ↑ | | 37.1 ± 2.1 | ↑ | 200000 (C)b | Sweat, bitter (F); cheese, rancid (D) |
| dimethylpyrazine | 10.0 ± 5.0 | | ↓ | 11.7 | | ↓ | | | | | | | | |
| 2-methylpiperazine | 31.9 ± 2.3 | 63.7 ± 2.2 | ↑ | 33.7 | 47.8 ± 4.5 | ↑ | | | | | | | | |

(Moll *et al.* 1994) (A);(Czerny *et al.* 2008) (B); (Guth 1997) (C); (Siebert *et al.* 2005) (D); (Boidron *et al.* 1988) (E); (Meilgaard 1975) (F); (Ribéreau-Gayon *et al.* 2006) (G); (Ferreira *et al.* 2000) (H); (Van Opstaele *et al.* 2011) (I); (Vanderhaegen *et al.* 2006) (J)

a Olfactory perception threshold in water, b Olfactory perception threshold in hydro-alcoholic solution, c Olfactory difference threshold in beer, d Olfactory threshold in model wine

↑ increase , ↓ decrease, = constant